

II. Contributions to our Knowledge of the Formation, Storage, and Depletion of Carbohydrates in Monocotyledons.

By JOHN PARKIN, M.A., Trinity College, Cambridge.

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PART I.—THE PRODUCTION OF STARCH IN THE FOLIAGE LEAVES OF MONOCOTYLEDONS.

SECTION I.—HISTORICAL SUMMARY.

MOST of our knowledge concerning assimilation has been gained from observations and experiments on Dicotyledons. SACHS'* work on the rôle of starch in the leaf referred to such Dicotyledons as *Helianthus*, *Phaseolus*, and *Cucurbita*, which are great starch-formers. In Monocotyledons, however, it is the exception to find starch in the mesophyll—just the reverse of what occurs in Dicotyledons.

BÖHM noticed as far back as 1856 that there are certain Monocotyledons—*e.g.*, *Asphodelus luteus*, *Allium fistulosum*, and *Orchis militaris*, the chloroplasts of which are incapable of manufacturing starch, and A. MEYER† in 1885 published his elaborate investigations on the occurrence of starch in various foliage leaves, and stated as a general conclusion that Dicotyledons store starch plentifully in their leaves, while Monocotyledons store little or none. He attempted to explain the absence of starch in these plants, and considered that it might be due to too rapid translocation of the assimilated products from the mesophyll as compared with the energy of assimilation. Experiments were made by exposing detached leaves to the most favourable conditions for assimilation, in order that the products might accumulate in the leaf, but the leaves of plants which did not form starch under ordinary circumstances also failed to do so here, with only two exceptions, *viz.*, *Hemerocallis flava* and *Muscari moschatum*. He considered, therefore, that the temporary reserve in the leaf assumes some other form. MEYER also attempted to investigate the nature and amount of sugars in such Monocotyledons as well as in some Dicotyledons, and obtained results indicating the interesting fact that the leaves of plants which store starch abundantly, contain comparatively little of the reducing and non-reducing soluble carbohydrates, whilst those which form no starch, such as *Iris germanica*, *Allium Cepa*, *Asphodelus luteus*, &c., accumulate large quantities of soluble reducing substances, probably hexoses. The appearance and disappearance of these carbohydrates seem to depend on the same causes which regulate the appearance and disappearance of starch in other leaves. Another important discovery made by MEYER is the occurrence of a fructose-producing carbohydrate, which he termed Sinistrin, in the leaf of *Yucca filamentosa*, and which is probably an Inulin.

SCHIMPER‡ confirmed MEYER's results as regards the greater quantity of sugar in leaves which do not normally produce starch. He found in the plants investigated that the amount of starch is in inverse proportion to the amount of glucose.

Further references to MEYER's important papers will be made later.

* SACHS, 'Bot. Zeit.,' 1862, 1864, 1884.

† A. MEYER, 'Bot. Zeit.,' 1885, Nos. 27–32.

‡ SCHIMPER, 'Bot. Zeit.,' 1885, p. 738.

SECTION 2.—THE PRESENCE OF STARCH DUE TO NORMAL ASSIMILATION.

In this section are described the results of the examination for starch in the foliage leaves of typical representatives of the Monocotyledons, to see what relation the presence or absence of starch bears to the character of the plant. Needless to say, this part of the work is by no means complete, though my survey of the three chief petaloid orders of temperate climes, viz., the Liliaceæ, Amaryllideæ, and Iridaceæ, is fairly extensive. As will be seen, the numerous examinations needed entail an enormous amount of labour, and a consequent restriction in the number of species investigated.

Bright sunny days, and mild for the time of year, were selected for obtaining material, in order that the conditions for assimilation might be as favourable as possible. The leaves were picked usually between 3 P.M. and 4 P.M., an early hour being chosen in order to avoid depletion through diminished light-intensity. The leaves selected were always those well exposed to direct sunshine, unless otherwise stated. Sometimes the plant required does not grow in a very sunny situation; in such a case the non-production of starch must be inferred with caution. The date, time of day, state of the weather, and temperature were all carefully noted when the leaves were picked. Another point to which attention was specially directed was the condition of the growth of the plant, whether before, at, or after its flowering period, since the age of the leaf might probably have something to do with its capacity for producing starch.

The leaves, when gathered, were at once put into alcohol to remove the chlorophyll; they can then be kept as long as desired before examination. In examining for starch, both macroscopic and microscopic methods were used with the same lot of material.

The former consisted in soaking the leaves in a solution of iodine in potassium iodide, which gives a general idea of the distribution and quantity of starch in the organ according as the yellow colour of a starch-free leaf deepens to a yellowish-grey, purplish, or black tint. The leaves were then carefully examined with the microscope. When the leaf is thin and membranous, the starch can be microscopically localised by mounting it whole, having previously treated it with iodized chloral hydrate solution to render it transparent; but most monocotyledonous leaves are too thick for this, and so the more troublesome method of section-cutting has to be resorted to. An additional examination of the same material, preserved in alcohol, is made at the same time, to see whether any inulin in the form of sphero-crystals can be detected: the results are considered in Part II. I have described below, in as concise a manner as possible, the examinations of foliage leaves made, the plants being arranged in their systematic order.

LILIACEÆ.

Uvularia grandiflora. Three examinations made.

- (1.) April 28, 3.30 P.M.* In full flower. A considerable amount of starch was detected macroscopically in the leaf and stem. The mesophyll is homogeneous, composed of about four layers of cells; the starch is chiefly confined to the two middle layers, none in the upper and hardly any in the lower layer. The greatest quantity of starch occurs in the cells surrounding the fibro-vascular bundles.
- (2.) May 19, 3.30 P.M. Finished flowering. Starch less abundant than in (1) but more so than in (3); most in the smaller upper leaves and upper parts of the stems.
- (3.) June 1, 3.30 P.M. Flowers wholly gone, leaving unripe capsules. Leaves quite green; did not show nearly as much starch as (1), being almost entirely confined to the cells around the vascular bundles, thus making them stand out black after treatment with iodine solution.

Veratrum album. May 27, 3 P.M. Flowers in bud. Starch in some abundance in the lower part of the leaf, in the inner mesophyll cells in considerable abundance, but none elsewhere.

Colchicum autumnale. May 19, 3.45 P.M. No starch in the general mesophyll, but some at the base of the lamina in a layer of cells round the vascular bundles.

Tulipa. Leaves from ordinary garden varieties. Examined three times when in flower. No starch found in the general mesophyll, but a little around the bundles at the base of the leaf and in the nodal regions. A plant taken up at 4.45 P.M., June 26, with its leaves still green, but which had finished flowering, showed no starch anywhere.

Fritillaria persica. One examination made on May 19. Plant finished flowering. Starch evident in small quantity round the bundles, especially in the basal part of the leaf.

Lilium tigrinum. Three examinations made.

- (1.) June 26, 5 P.M. Some time before flowering. Showed plenty of starch in the leaf, its distribution being similar to that of *Uvularia*. The mesophyll consists of a palissade layer and about six other layers. Starch chiefly confined to the three middle layers, and here the chloroplasts became quite black with iodine.
- (2.) August 1, 4.15 P.M. Coming into bloom. Starch quite pronounced, but not so much as in (1), though similarly distributed in the mesophyll.
- (3.) September 4, 3 P.M. Finished flowering. Tips of leaves turning yellow. No starch in the lamina except at the very base around the vascular bundles.

Leaves of *Lilium Martagon*, growing somewhat in the shade, were also examined on several occasions, and showed no starch.

Scilla sibirica. Two examinations were made of leaves from plants with their flowers fading; they showed no starch in the mesophyll. In one case the whole plant was investigated and no starch found at all above the sheath of the leaf, which functions as a storage organ (scale).

Hyacinthus orientalis. Plants in a greenhouse. Two examinations of the upper assimilating part of the leaf revealed no starch in the mesophyll, but some occurred in a layer of cells around the vascular bundles in the lower part, about 20 centims. from the apex.

Allium Cepa.

- (1.) Seedlings taken up 10 A.M., June 1. Possessing two leaves, the older one evidently being the first foliage leaf produced after the cotyledon. Both leaves showed starch around the vascular bundles for 2 to 3 centims. upwards from the sheath; starch also throughout the sheaths of the leaves around the bundles.
- (2.) First year plants taken up 3.30 P.M., May 17, further advanced in growth than (1), had no starch at all above the sheaths.
- (3.) Second year plants, taken up at the same time as (2), possessed no starch.

* The dates refer to the time of picking.

Allium Schoenoprasum.

- (1.) May 9, 3.30 P.M. Flowers in bud. In the upper assimilating portion of the leaf no starch was found; but in the lower part above the sheathing base starch was seen in the parenchymatous cells bordering on the vascular bundles, especially around the phloëm.
- (2.) May 15, 10 A.M. No starch in the leaf above the sheath.
- (3.) May 27, 3.15 P.M. In flower. Just a trace of starch was found above the sheath in the cells at the junction of the xylem and the phloëm of each bundle, but not nearly so evident as in (1).

Triteleia uniflora.

- (1.) April 10. Just commencing to bloom. No starch in the mesophyll.
- (2.) April 18. No starch.
- (3.) May 9. Past flowering for some time. A few minute granules of starch found in the chloroplasts of two or three cells around the vascular bundles in the lower part of the leaf.
- (4.) May 20. A little starch in the cells around the bundles in the lower part of the leaf.

Agapanthus umbellatus. In the upper assimilating portions of the leaves from a plant in a greenhouse, on November 19 (bright sun all day), starch was present in a few of the cells abutting on the vascular elements at a distance of 16 centims. from the apex of the leaf downwards.

Anthericum liliago. Two examinations made about end of May showed starch only at the base of the leaf around the bundles.

Asphodelus luteus.

- (1.) May 9. No starch in the mesophyll except at the base of the leaf, where it occurs in two to three cells bordering on the bundles.
- (2.) May 19. Not yet in flower. Starch present around the bundles in the lower part of the leaf.
- (3.) June 1. In flower. No starch except as in (2).

Aloe plicatilis. The fleshy leaves of a plant in a greenhouse showed starch in the cells around the bundles throughout the leaf, but none in the mesophyll proper.

Funkia ovata.

- (1.) May 27. Leaves from plants as yet showing no signs of flowers exhibited traces of starch in the lamina; after iodine the larger veins stood out blue and the marginal part of the lamina bluish. In the petiole a fair amount of starch occurred in the single arc of cells round the phloëm of each bundle.
- (2.) June 26, 3.15 P.M. Leaves taken from a different group of plants bearing flower-buds, growing in an easterly aspect and so obtaining the morning sun, showed no starch macroscopically; but a very little was revealed by the microscope round the main bundles, increasing in amount down the petiole, at the base of which it occurs in a layer of cells bordering the phloëm.

Convallaria majalis.

- (1.) May 9. In flower. No starch occurred in the expanded part of the leaf, but there was a little in the cells around the bundles in the sheath.
- (2.) May 19. In flower. Similar to (1).
- (3.) June 1. After flowering. Very little starch evident; most conspicuous near the apex, but by no means throughout the mesophyll.

Polygonatum multiflorum.

- (1.) May 19. In flower. Starch apparent only around the veins of the leaf.
- (2.) May 27. Finished flowering. No starch except at the very base of the leaf where the veins converge, in a layer of cells around the bundles.

Paris quadrifolia. May 9, 3.30 P.M. From a plant in flower, and somewhat in the shade. A certain amount of starch indicated macroscopically in patches. Sections show it in all the mesophyll layers except the one beneath the upper epidermis. The cells around the bundles are mostly devoid of starch.

Asparagus officinalis.

- (1.) June 1. Not in flower. Macroscopically a little starch apparent in the phylloclades.
- (2.) June 26. In bloom. Only a little starch.
- (3.) July 11. A small amount of starch.
- (4.) July 20. Sprigs of plants with full-sized but still green berries. No starch visible, except indistinct traces occasionally at the tips of the phylloclades.

Smilax herbacea. June 1. Recently grown stems, as yet showing no signs of flowers. In the lower leaves starch was confined to one or two layers of cells round the vascular bundles of the principal veins.

PONTEDERIACEÆ.

Pontederia cordata. Leaves from a plant growing in a tank in a greenhouse, in flower. Showed starch here and there in the lamina.

AMARYLLIDEE.

Galanthus nivalis.

- (1.) March 2, 3 P.M. In flower. A little starch was made out microscopically in the large parenchymatous cells surrounding the main bundles; this was traced upwards to where the lysigenous spaces begin to appear and the leaf widens. Starch also occurred in the peduncle, being here more especially visible in the cells at the junction of the xylem and phloëm, and could be traced up to within 3 centims. of the floral spathe.
- (2.) March 6. Starch as in (1).
- (3.) March 14. No starch in the leaf.
- (4.) March 30. Flowers just gone. No starch above the leaf sheaths (scales of bulbs).
- (5.) April 16. As in (4).

Leucojum aestivum.

- (1.) May 9. In flower. No starch in the general mesophyll, and only in small amount in the lower part of the leaf near the bundles.
- (2.) July 23. As in (1).

Narcissus poeticus. Several examinations showed no starch in the upper assimilating portion of the leaf, but it occurred round the vascular bundles in the lower part.

Narcissus pseudo-narcissus (var. *plenus*). Three examinations.

- (1.) March 30. Flower not expanded. No starch in the ordinary mesophyll, but about 15 centims. from the apex of the leaf it begins to be visible in cells near the main bundles, becoming more apparent lower down, and extending at last to a complete layer of cells around all the bundles, even the small ones.
- (2.) April 16. No flower. Starch as in (1).
- (3.) May 10. Starch around the bundles in the leaf was traced for 23 centims. upwards, the whole length of the leaf being 51 centims.

Narcissus jonquilla. One examination of the upper half of the leaf showed no starch.

Narcissus Tazetta.

- (1.) May 11. Flowers faded some time. No starch anywhere.
- (2.) May 19. No starch anywhere.

Alstræmeria aurea.

- (1.) May 27. Plant in full leaf, but devoid of flowers. Starch was present in some leaves, especially those near the apex of the stem, which assumed a dark blue colour with iodine; the upper parts of the stem also turned dark blue. Below the twist in the leaf starch occurred in a layer of cells round each of the bundles.
- (2.) July 20. Leaves from different plants in an eastern aspect, and in flower. A little starch here and there in the mesophyll, chiefly near the tips of the leaves.
- (3.) August 1. Leaves from the same plants as (2). After flowering, showed no starch at all.

BROMELIACEÆ.

Ananassa sativa. Picked on March 2 from a stove, at 3.15 P.M. Starch fairly abundant throughout the mesophyll.

IRIDACEÆ.

Iris. Five species were examined, *I. benensis*, *I. ochroleuca*, and *I. plicata* having the characteristic ensiform leaves.

Iris orchidoides possesses cauline leaves.

Iris benensis.

(1.) April 28. Flowers fading. No starch in the upper part of the leaf.

(2.) May 9. Starch only in the sheathing (basal) part of the leaf.

I. ochroleuca. June 26, 5 P.M. The upper parts of the leaves of plants in flower showed no starch.

I. plicata.

(1.) May 27. Flower buds not quite ready to expand. Starch only in the lower part of the leaf, none in the assimilating portion.

(2.) June 1. No starch.

I. Xiphium.

(1.) May 9. Not yet in flower. Starch present only in the base of the leaf, especially round the bundles.

(2.) June 1. Flowers not yet expanded. No starch anywhere.

Schizostylis coccinea. August 1, 4 P.M. The upper parts of the leaves, from plants in flower, gave clear indications of starch all over, especially at the tips.

Crocus vernus.

(1.) March 2. In flower. A trace of starch in the cells bordering on the main bundles, where the leaf commences to narrow.

(2.) March 24. Finished blooming. No starch.

(3.) May 9. No starch.

Crocus aureus. Similar to *C. vernus* as regards starch.

Gladiolus (garden varieties).

(1.) June 26. Flower buds not yet visible. Only the upper parts of the leaves were examined, and showed no starch.

(2.) July 19. A very little starch round the veins throughout the leaf.

(3.) August 1. Flower buds showing. A little starch here and there in the mesophyll.

(4.) September 4. Finished flowering. No starch.

CANNACEÆ.

Canna indica.

(1.) May 9. In flower, in a conservatory. Starch was visible in the chloroplasts throughout the mesophyll, and was also conspicuous around the vascular bundles of the midrib.

(2.) June 1. In full bloom. Starch as in (1).

ORCHIDACEÆ.

Listera ovata. One examination made of entire plants taken up when flowering, June 26, 4.30 P.M. No starch in the leaf-mesophyll at all, but in the stem below the leaf-insertions it was present in a more or less regular layer of cells outside the stele, gradually diminishing towards the root, and disappearing at the very base.

Another examination, made on July 13, showed no starch in the leaf.

Orchis maculata.

(1.) June 26. In full bloom. Plants growing in a slightly shaded place showed a certain amount of starch in some parts of the leaves, especially in the narrower ones.

(2.) August 6. Leaves from rather shaded plants, with unripe capsules, showed no starch.

Orchis latifolia. May 9. Leaves from a plant somewhat in the shade had no starch in the mesophyll, but a few cells around the main bundles at the base of the leaf possessed a little.

Cattleya Bowringiana. Leaves from a plant in bloom in an orchid house showed a little starch throughout.

COMMELYNACEÆ.

Tradescantia virginica.

(1.) May 27. Just commencing to flower. A certain amount of starch in the leaf, chiefly confined to the middle part of the mesophyll, and absent from the two outer layers.

(2.) July 20. Well advanced in flower. Leaves from different plants not so well exposed to the sun, showed a very little starch in the basal parts near the veins, and traces elsewhere.

(3.) Leaves picked from the same group of plants as (2), but later, showed no starch anywhere.

TYPHACEÆ.

Sparganium ramosum.

(1.) July 20. Pollen just being shed. The upper parts of the long linear leaves contained starch throughout.

(2.) August 6. Ovaries somewhat swollen. Starch as in (1).

ARACEÆ.

Arum maculatum. The leaves examined three times in the spring showed starch only in the veins of the midrib towards the base of the lamina, and restricted to the cells just outside the phloëm.

Richardia africana.

(1.) Leaves from a plant not yet in flower—in temperate house—contained a fair amount of starch in the mesophyll, but not throughout.

(2.) From a plant in flower. Starch in small quantity in the mesophyll.

JUNCACEÆ.

Juncus communis.

(1.) June 26. In the upper well-exposed parts of the cylindrical leaves starch was very evident, and in the mesophyll, except in a layer or so of cells beneath the epidermis.

(2.) August 6. Starch present, but less in amount than in (1).

CYPERACEÆ.

This natural order has not been investigated to any great extent, only the leaves of the common greenhouse foliage plant, *Cyperus alternifolius*, having been examined. These contained a considerable amount of starch, especially in the lower halves, and the stem bearing them turned almost black with iodine.

GRAMINEÆ.

Avena sativa (cultivated).

(1.) June 22, 4 P.M. Leaf-blades from plants about a foot or so in height, and with no signs of the ears, showed a moderate amount of starch.

(2.) July 5, 4.45 P.M. In full ear, but the pollen not yet shed. No starch in the blade or sheath

but clearly visible near the junction of the blade with the sheath, the veins here standing out blue after iodine.

- (3.) August 1, 3.40 P.M. Spikelets still green. A little starch in the same situation as (2).
- (4.) August 21, 3.30 P.M. Leaves withering and spikelets turning yellow. Portions of the stem still quite green showed no starch.

Triticum vulgare.

- (1.) June 22, 4 P.M. Leaf-blades of plants which had just shed their pollen. Starch present in about the same proportions as in *Avena* (1).
- (2.) July 5, 4.45 P.M. Finished flowering and the leaves beginning to dry up at their tips. Starch only at the junction of the blade and sheath around the bundles.
- (3.) August 1, 3.40 P.M. Green parts of the stem contained no starch.

Phleum pratense.

- (1.) June 26, 4 P.M. Pollen not yet shed. No starch occurred, except a little above and below the node and in the swollen base of the sheath, in a layer of cells around the bundles.
- (2.) July 5, 4.45 P.M. In flower. No starch at all.
- (3.) July 11, 4.45 P.M. In ear but not yet in flower. Just an indication of starch in some of the blades.
- (4.) August 1, 3.45 P.M. Leaf-blades still green. Starch present only in the basal swollen part of the sheath.

Saccharum officinale. Leaves taken from a greenhouse showed a little starch throughout the leaf-blade, confined to a single row of cells round all the bundles.

Bambusa siamensis. From a palm-house. Showed starch here and there in the leaves.

ALISMACEÆ.

Alisma Plantago.

- (1.) July 20. With flower-buds. The leaves contained an appreciable quantity of starch throughout the mesophyll, but none in the chloroplasts of the epidermal cells.
- (2.) August 6. In full bloom. Starch as in (1).

The following showed no starch:—*Fritillaria imperialis*, *F. Meleagris*, *Erythronium Dens-canis*, *Scilla nutans*, *Ornithogalum byzantinum*, *Allium Porrum*, *A. sativum*, *Hemerocallis fulva*, *H. flava*, *Phormium tenax*, var. *atropurpurea*, *Cordyline superbens*, *Iris orchidoides*.

General Remarks.

Of the plants thus examined only relatively few seem to have elaborated starch to any considerable extent in their mesophyll after a favourable day's illumination; but the following are fairly conspicuous starch-formers out of about seventy investigated:—*Uvularia grandiflora*, *Lilium tigrinum*, *Pontederia cordata*, *Alstræmeria aurantiaca*, *Ananassa sativa*, *Schizostylis coccinea*, *Canna indica*, *Cattleya Bowringiana*, *Tradescantia virginica*, *Sparganium ramosum*, *Richardia africana*, *Juncus communis*, *Cyperus alternifolius*, *Alisma Plantago*.

On comparing these results with those mentioned by MEYER,* they are found to agree pretty closely. He claims for the Colchicaceæ a moderate quantity of starch in the foliage leaf. My observations show a considerable amount in *Uvularia*, and a

* MEYER, *loc. cit.*, 1885.

little in *Veratrum* and *Colchicum*. *Uvularia* sends up branched serial stems with numerous medium-sized leaves, while the other two species have radical foliage leaves, which are larger and not nearly so numerous; it is possible that this difference in foliar habit may account to a large extent for the much greater production of starch in *Uvularia*.

Of the species belonging to the tribe Tulipeæ, MEYER mentions *Lilium bulbiferum* as containing a moderate amount of starch. The examinations of the leaves of *Lilium tigrinum* similarly reveal starch. The genera *Fritillaria* and *Erythronium* are devoid of starch, and *Tulipa* has very little. The latter is a plant with an aerial stem bearing only a few leaves, while *Lilium* has one with many, and is the starch-producer, as in the case of *Uvularia*. *Fritillaria*, especially the species *F. imperialis*, however, seems to contradict this, as it has a very leafy stem. In this connection, however, there is a further point to be taken into consideration, viz., the temperature. *F. imperialis*, as well as *F. Meleagris*, are fairly early spring bloomers, while *Lilium* flowers in the summer; thus the temperature is much higher for the latter, and hence the rate of assimilation may be quicker. The days on which leaves of *F. imperialis* were picked had a maximum shade temperature below 15°, while those for *L. tigrinum* had a corresponding one of about 21°. Moreover, in the case of *F. persica*, a little starch did occur in the leaves, and these were picked later in the spring, with a temperature of 18°·3. *Erythronium* is also an early spring flowering plant, and, although similar to *Tulipa* in foliar organs, it showed no starch; this, also, may be due to too low a temperature, the leaves of *Tulipa* being picked either late in the spring or from a greenhouse.

Concerning the tribe Hyacinthæ, MEYER says that a little starch occurs in the leaf of *Ornithogalum caudatum*, none in *Scilla maritima*, *S. hyacinthoides*, *Muscari racemosum*, *M. moschatum*, and *Ornithogalum comosum*. I find no starch in the upper exposed parts of the foliage leaves of *Scilla*, *Hyacinthus*, *Muscari*, and *Ornithogalum*, although in *Hyacinthus* starch was present in the lower part of the lamina, but not in that of *Scilla sibirica*. The former was in a greenhouse and the latter growing in the open, whence the differences in temperature may again account for the occurrence of starch in the one and its absence from the other. It may be concluded that the Hyacinthæ are less productive of starch in their foliage leaves than the Tulipeæ. There are two marked differences in the leaves of these two tribes. The Hyacinthæ possess wholly radical leaves, linear in outline, while the Tulipeæ have broader cauline ones, lanceolate or ovate in shape.

With respect to the tribe Allieæ, the genus *Allium* has long been noted as not producing starch in the foliage leaf. MEYER* mentions six species examined by him as being all starchless. RENDLE† has shown, however, that the leaves of very young plants of *Allium Cepa* contain starch in the elongated parenchymatous cells bordering

* MEYER, *loc. cit.*

† RENDLE, 'Annals of Botany,' vol. 2, 1889.

on the vascular bundles, when picked in the afternoon of a warm sunny day. He rarely found starch in the leaves of older onions. I have to a certain extent confirmed his results, for I find starch in the green upper part of the leaves of seedling onions, even when picked in the morning. Older plants of *A. Cepa*, as well as of *A. Porrum*, failed to show starch. *Allium Schaenoprasum* had a little starch in the lower part of the lamina. *Triteleia* and *Agapanthus* also show starch in a similar situation. The Allieæ are thus like the Hyacintheæ as regards starch-formation, and both possess radical leaves. The genus *Allium* is somewhat exceptional, and appears to furnish the extreme case of non-production of starch; it is shown further on to be practically the only one which forms no starch in the guard-cells of the stomata.

Concerning the tribe Anthericeæ, MEYER gives *Asphodelus luteus*, *Anthericum ramosum* and *A. lilago* as without starch; but I find that in both genera starch is present in small quantities in the lower part of the lamina. The leaves in the two plants are partly radical and partly cauline, as well as numerous and linear.

Among the Hemerocallideæ, MEYER mentions *Hemerocallis flava* and *H. fulva* as being without starch, and *Funkia* as having a moderate amount, with which my results agree in the main. *Hemerocallis* has broadly linear radical leaves; this difference may account to some extent for the starch-formation in *Funkia*.

The Convallarieæ are not referred to in MEYER's paper. *Convallaria* has some starch, and *Polygonatum* scarcely any; this seems to contradict what precedes, because the former has few radical leaves and the latter many cauline ones; *Convallaria* however stores starch in its rhizome while *Polygonatum* does not.

MEYER asserts that Amaryllidaceæ for the most part contain little starch. He examined the leaves of *Narcissus poeticus*, *N. odoratus*, *N. biflorus*, *Leucojum æstivum*, and *Amaryllis undulata*, and found them starch-free; this agrees with my observations, except that I find starch occurs in the lower part of the lamina of these plants around the bundles. *Alstræmeria* contains a fair amount of starch, and has an aerial stem with numerous leaves, like *Uvularia* and *Lilium*.

With respect to the Iridaceæ, MEYER concludes that they store very little starch in the leaf, most occurring in *Tritionia*, less in *Gladiolus*, and very rarely any in the assimilating parenchyma of *Iris florentina*, *I. sibirica*, *I. graminea*, *I. germanica* and *I. pallida*; thus agreeing with the foregoing observations.

The Commelynaceæ are put down by MEYER as containing a moderate quantity of starch, and the only plant I examined, *Tradescantia virginica*, had some starch in its leaf.

He also mentions the Juncaceæ as storing much starch, and the Alismaceæ as having a moderate quantity in their leaves.

The Gramineæ he found to contain much less starch than the Cyperaceæ, whilst the Dioscoreaceæ store a quantity *e.g.* *Tamus communis*, *Dioscorea villosa* and *D. Batatas*. I have not as yet examined any of this order.

The shape, venation and mode of attachment of the leaf thus appear to have some

connection with starch-production. Broadly lanceolate, ovate, sagittate and petiolate leaves seem more apt to produce visible starch than linear sessile ones; cauline leaves also show more than radical ones, and reticulately veined more than parallel veined ones, *e.g.* the Dioscoreaceæ. This probably depends on the ratio between the rate of assimilation and the rate of translocation, the latter taking place more quickly in one class of leaves than in the other.

Another point which strikes one in a general survey of the occurrence of starch in monocotyledonous foliage leaves is that the production of this carbohydrate is most marked in aquatic plants. The Cyperaceæ and Juncaceæ are greater starch-formers than the Gramineæ, and are much more aquatic in habit. *Schizostylis coccinea*, one of the Iridaceæ, is a moisture-loving plant, and accumulates starch in its foliage leaves. *Sparganium* is a marsh plant, and produces starch in considerable amount. The Alismaceæ and *Hydrocharis*, *Elodea* and *Iemna*, decided starch-producers, are all water plants. Most of these plants have no special reserve-organs, and this may have some bearing on the occurrence of starch in the leaf. A plant with a tuber or bulb in which to store carbohydrates, may rarely have sufficient concentration of sugar in the leaf to necessitate starch deposition there, while a plant without such an organ must store the excess in the mesophyll.

Another question to which particular attention has been paid in the examination of monocotyledonous leaves is how far the age of the leaf has anything to do with its capacity for forming starch. No conclusive evidence has been obtained on this point, but certain facts suggest the idea that in some Monocotyledons at least the power of the chloroplasts to elaborate starch diminishes as the age of the leaf increases; *e.g.*, the cases of *Uvularia grandiflora*, *Lilium tigrinum*, *Alstræmeria aurea*, *Iris plicata* and *I. Xiphium* and *Avena sativa*. *Allium Cepa* also contains starch in its young state, but not afterwards.

SECTION 3.—THE STARCH OF THE STOMATAL GUARD-CELLS.

SACHS* pointed out that the guard-cells still contain starch after the rest of the leaf is depleted, and mentioned *Tropæolum* as containing starch in its stomata after 48 hours in the dark.

A. MEYER† incidentally mentions the presence of starch in the guard-cells as well as round the vascular bundles in the leaf of *Asphodelus luteus* after insolation in an atmosphere containing 3 per cent. carbon dioxide, whereas species of *Allium* showed no starch anywhere under similar conditions.

HORACE BROWN has called attention to the isolation of the guard-cells from the mesophyll cells, as a factor in regard to the long retention of starch in their chloroplasts.

* SACHS, 'Bot. Zeit.,' 1862.

† MEYER, 'Bot. Zeit.,' 1885.

In the foregoing investigations on the starch in Monocotyledons, special attention was paid to the stomatal starch, and in the plants examined it has been found almost invariably present in all of them except in species of *Allium*. The Tulipeæ contain a large amount, the Hyacintheæ a moderate quantity, *Triteleia* (Alliæ) only a little, and the *Anthericeæ* and *Convallariaceæ* plenty. In the Amaryllideæ, *Narcissus* presents a moderate quantity and *Galanthus* a little. *Sparganium* has a pair of subsidiary cells to the stoma, and these, as well as the epidermal cells, contain chlorophyll; but the guard-cells seem to be the only ones which have starch. *Alisma* has chloroplasts similarly situated, and in this case starch was apparent in the subsidiary cells as well as in the guard-cells, but not in the general epidermal cells. The Juncaceæ, Cyperaceæ, and Gramineæ have very small, narrow guard-cells, and it is difficult to see whether they contain starch or not; on some occasions, however, it was quite evident, in others uncertain.

There are also indications that as the leaf grows older the guard-cells show less starch. *Alstræmeria*, on May 27, had its guard-cells full of starch; on July 20, only a moderate quantity; and on August 1, mere traces. *Gladiolus*, on June 26, had a little in its stomata, and on September 4 I failed to find any. *Orchis maculata* possessed a considerable amount on June 26, and on August 6 some stomata were without any, others with a fair quantity. *Tradescantia*, on May 27, had a moderate amount, and on July 20 a trace; but on September 4 none. The reason, I think, why the starch disappears is that the chlorophyll is destroyed. A leaf of *Orchis maculata*, examined on August 6, showed some of the guard-cells with brownish, not green, contents, and in these no starch could be rendered visible.

How is it the guard-cells of *Allium* have no starch?

With regard to *Allium Cepa* and *A. Porrum*, it might be thought that the inability to manufacture starch has extended even to the chloroplasts of the guard-cells; but *Scilla nutans* is almost, if not quite, as poor a starch-producer as the onion, and yet its stomata contain plenty. Again, *Allium Schænoprasum* usually possesses starch around the vascular bundles in the lower part of the leaf-lamina, and yet its guard-cells contain no starch. In examining the leaves of *Allium*, preserved in alcohol, I was struck with the absence of anything in the guard-cells resembling chloroplasts, and was thus led to examine the fresh leaf by stripping off portions of the epidermis and looking at them under a high magnifying power. No chloroplasts could be recognized, nor could I certainly detect any green colour in the guard-cells; whilst on repeating the observations with other Monocotyledons, such as *Lilium*, *Hyacinthus*, *Triteleia*, *Alstræmeria*, &c., the green chloroplasts were quite in evidence. Thus it seems that the absence of starch in the stomata of *Allium* may be due to the want of plastids. If any chlorophyll be present, it must be in very small quantities. Examinations of the following species have revealed no starch or chlorophyll in their guard-cells: *Allium Cepa*, *A. Porrum*, *A. sativum*, *A. Schænoprasum*, *A. fistulosum*,

A. Neapolitanum, *A. Babingtoni*, and *A. obliquum*. Sometimes the guard-cells possess small refractive granules.

The guard-cells in which it occurs retain their starch very tenaciously. Some detached leaves of *Narcissus poeticus* were put in the dark and left for seven days, yet starch was still present; even after fifteen days it had not wholly disappeared, but beyond this time the leaves turn yellow and wither. It would seem that it is almost impossible to make the starch vanish from the guard-cells of the living leaf.

It is natural to suppose, and no doubt is usually the case, that the starch is produced directly in the guard-cells by the assimilative activity of their chloroplasts. The starch, however, need not necessarily be autochthonous. Bulbs of *Narcissus poeticus* were grown in the dark, and when the leaves were mature, they were examined, and their stomata showed a moderate quantity of starch. Also plants of *Galanthus nivalis*, similarly grown, showed a little starch in the guard-cells of the upper mature part of the leaf, but not in the lower, thus evincing a deposition of starch in the guard-cells only when the stomata are fully formed and functional.

SECTION 4.—FORMATION OF STARCH IN DETACHED LEAVES OF SOME MONOCOTYLEDONS WHEN EXPOSED TO SUNLIGHT.

One of the most probable explanations of the non-production of starch in foliage leaves exposed to favourable conditions, is that the amount of sugar in the cell-sap of the mesophyll does not reach a sufficient degree of concentration to necessitate the deposition of part of it as starch. This idea seems to a large extent warranted now that SACHS' notion, that all the assimilated carbohydrate has to pass through the starch stage, is untenable.

SCHIMPER* in his paper on the formation and translocation of carbohydrates in the foliage leaf concludes that starch-formation results when the sugar (which he regards as glucose) reaches a certain degree of concentration, varying for different species. Hence, in the case of plants which do not normally produce starch in their leaves, it would seem desirable to see if this carbohydrate could be produced in them when detached, placed in water, and exposed to sunlight, thus preventing the products of assimilation from escaping and increasing the percentage of sugar in the cell-sap till it reached the point to bring about the deposition of starch.

A. MEYER† experimented in this manner, and, to make the conditions more favourable, exposed the cut leaves, not in the ordinary atmosphere, but in one containing 3 per cent. carbon dioxide, which GODLEWSKI‡ had shown increased the rate of assimilation considerably. Species of *Allium* and *Anthericum ramosum*, thus exposed for two days, produced no starch, while *Asphodelus luteus* exhibited it round the bundles.

* SCHIMPER, 'Bot. Zeit.,' 1885.

† MEYER, 'Bot. Zeit.,' 1885.

‡ GODLEWSKI, 'Flora,' 1873.

Muscari moschatum, *Iris germanica*, and *Hemerocallis flava* also produced some starch. These confirm BÖHM's* previous results, who found that *Galanthus nivalis*, *Hyacinthus orientalis*, and *Ornithogalum umbellatum* remain starch-free after one day's insolation in 5 per cent. carbon dioxide. *Iris germanica*, he says, ordinarily starch-free, often (but by no means always) contains starch in such an atmosphere. On the whole, then, it seems that starch is formed scarcely at all, or with difficulty, under such favourable conditions; so much so that MEYER expressed the opinion that the carbohydrate took on some other form.

My experiments performed in this direction are as follows:—In the leaves used, care was taken to remove a part from the base of each before insolation and to ascertain microscopically that it contained no starch, even round the bundles; for, if starch-free here, it might be fairly assumed that none occurred above; and so any starch resulting after the experiment would be due to the insolation. The experiments were carried out in the ordinary atmosphere, unless otherwise stated, and the cut ends of the leaves put in ordinary water.

Erythronium Dens-canis. Picked the leaves 10 A.M. March 27, and exposed them to the sun in a cool greenhouse. After six days no starch; after nine days, starch occurred in the mesophyll-cells around the main bundles; after eleven days, starch, similarly situated. Leaves now commencing to wither. The weather during the time was fairly sunny.

Hyacinthus orientalis. The upper parts of the leaves exposed on April 5 at 9 A.M., and examined at intervals till April 20, when they showed signs of withering. No starch found anywhere except in the stomata.

Hemerocallis flava. The upper parts of the long leaves were exposed to the sun from 11 A.M. May 15, to June 7, and then examined; traces of starch in the mesophyll here and there, but not in any quantity.

Galanthus nivalis.

- (1.) The upper parts of the leaves were put in the sun in an unheated greenhouse on March 24, 10.30 A.M., and left till April 4, being examined at intervals. No starch was found anywhere except in the guard-cells.
- (2.) Leaves similarly exposed from March 30 to April 8. Showed no starch.
- (3.) From April 5 to 17. No starch.

Narcissus poeticus (var. *ornatus*). Upper parts of the leaves of plants in full bloom were put in a heated greenhouse from February 24 to March 12. A moderate amount of starch occurred around the bundles.

N. poeticus (out-door variety).

- (1.) Leaves taken from plants with flower-buds just visible on March 30th, at 10.30 A.M., were insolated in a cold greenhouse till 5 P.M. April 12. A macroscopic examination of them with iodine solution gave a greyish-yellow tint, indicating a little starch; this microscopically was shown to be situated throughout the spongy mesophyll, but not in the palissade-cells, and was most conspicuous round the vascular bundles.
- (2.) Leaves from plants which had finished flowering were exposed from May 15 to May 25. Starch was present in the cells round the bundles extending a little way out, but not in any great quantity.

* BÖHM, 'Bot. Zeit.,' 1883.

Narcissus Pseudo-Narcissus. Insolated from April 5 to April 20. Starch was seen in small quantity throughout the mesophyll, except in the palissade-cells.

Crocus vernus. The leaves were exposed in a cold greenhouse from March 30 to April 12. Some parts went nearly black with iodine, and hence showed abundance of starch.

Iris flavescens. The upper part of the ensiform leaves were picked 3 P.M. May 6, from plants not yet in flower. Part were put outside and part in a greenhouse in the same situation. Examined on May 10 (four days), no starch in the mesophyll; examined the remainder on May 17 (ten days), a fair amount of starch round the bundles and in some of the outer mesophyll cells, but not throughout. The one in the greenhouse seemed to have more starch, evidently due to a higher temperature.

With *Colchium autumnale*, *Fritillaria imperialis*, *Scilla sibirica*, *Muscari botryoides*, *Asphodelus luteus*, *Funkia ovata*, *Polygonatum multiflorum*, *Iris orchidoides*, *I. benensis*, *I. plicata* and *I. Xiphium*, negative results only were obtained.

Few experiments were made with cut leaves in atmospheres containing higher percentages of carbon dioxide. I might, however, record two somewhat negative experiments with the leaves of *Narcissus poeticus*. The upper halves of the leaves, placed at 12 noon on February 24 in 5 and 10 per cent. carbon dioxide respectively, and left till 3.30 P.M. February 27 (three and a-half days) in a heated greenhouse, obtaining a fair amount of sunshine, showed no starch in the mesophyll.

A similar experiment, conducted in 10 per cent. and 2 per cent. carbon dioxide and left five days, revealed starch in the cells surrounding the vascular bundles of the 2 per cent. ones, in the 10 per cent. very little or none.

General Remarks.

In such experiments as the above, one is struck with the difficulty of making isolated leaves produce starch, and the length of time needed before it makes its appearance.

In the case of *Erythronium*, no starch was observable after six days' insolation.

„	„	<i>Narcissus</i>	„	„	„	seven	„	„
„	„	<i>Iris flavescens</i>	„	„	„	four	„	„

Thus nine or more days appear to be required. This seems contrary to what might be expected, if the leaf has the power to form starch at all. Three to four days one would have thought sufficient to increase the products of assimilation up to the starch limit.

BROWN and MORRIS* found that the production of starch in cut leaves of *Tropæolum* did not go on with the same energy as it did when the leaves were still attached to the plant, whereas the sugars increased greatly over those in the attached leaves.

SECTION 5.—THE FORMATION OF STARCH IN LEAVES OF MONOCOTYLEDONS FLOATED ON SUGAR SOLUTIONS.

Another way to test a leaf's capacity of producing starch is to supply it with

* BROWN and MORRIS, "Chem. and Phys. of Foliage leaves," 'Jour. of Chem. Soc.,' 1893.

solutions of sugars. Experiments in this direction have been made by several observers.

BÖHM* was the first to try them. He employed a 20 per cent. solution of cane-sugar, and found after eight to ten days that species of *Allium* and *Asphodelus luteus* were starch-free, while *Galanthus nivalis*, *Ornithogalum umbellatum*, *Hyacinthus orientalis*, *Iris germanica*, and *Veratrum nigrum* contained starch. There was no marked difference in the production of starch between the temperatures 10° and 20° C.

A. MEYER† confirmed the formation of starch in *Iris germanica* under these circumstances, and alter‡ published the results of his elaborate experiments, in which, however, only dicotyledonous leaves were used. He used, as a rule, 10 per cent. solutions of glucose, fructose, and galactose, at a temperature of about 15°, and found great differences in the sugars, as regards furnishing starch, according to the nature of the plant. The Sileneæ form starch fairly readily from galactose, while other plants fail to do so. Fructose is usually more favourable to starch-formation than glucose, especially in the Compositæ, in which order the latter affords mere traces. The Oleaceæ are somewhat exceptional in preferring glucose. He also experimented with cane-sugar, and found that this sugar, as a rule, furnished starch more readily and in greater abundance than the other carbohydrates, except in the Sileneæ, Oleaceæ, and some Compositæ. Mannite contributed largely to starch-production in the Oleaceæ. *Cacalia suaveolens*, a Composite, formed starch easily and copiously from a 10 per cent. glycerine solution, while *Dahlia variabilis* and *Beta vulgaris* furnished a trace, and other plants none.

ACTON§ performed a series of experiments by supplying to the roots and cut stems of plants solutions of various substances, including carbohydrates, with the primary object of seeing whether any aldehydic body could lead to starch-formation in the foliage leaf—but with only negative results. However, a few of his incidental results are of interest here. On supplying roots of plants with 1 to 5 per cent. solutions of glucose and cane-sugar, starch appeared in the leaves after five days. He concluded that glucose is more readily taken up by the roots than cane-sugar from 5 per cent. solutions. Glycerin supplied to the roots produced starch, but not if over 10 per cent. in strength. He also mentions that a 1 per cent. inulin solution produced starch in the leaves of *Acer* and *Cheiranthus* after five days, when given to the roots. The plants used throughout his experiments were chiefly Dicotyledons.

BROWN and MORRIS|| found that excised embryos of the barley grain grow best in solutions of cane-sugar, the optimum concentration being 3·5 per cent. to 4 per cent.

* BÖHM, 'Bot. Zeit.,' 1883.

† MEYER, 'Bot. Zeit.,' 1885.

‡ *Ibid.*, 1886.

§ ACTON, 'Proc. Roy. Soc.,' 1890.

|| BROWN and MORRIS, "Germination of Barley," 'Jour. Chem. Soc.,' 1890.

Invert-sugar, glucose, fructose, and maltose, although adding very appreciably to the nourishing of the young plant, were not as beneficial as cane-sugar. Galactose acted very feebly, and lactose not at all. Glycerin was of slight value.

SAPOSCHNIKOFF* reckoned that the maximum concentration of sugar in the cell-sap in the leaves of *Vitis vinifera* and *V. labrusca*, and *Rubus cæsius* and *R. fruticosus*, approaches that of a 10 per cent. glucose solution. A 2 per cent. glucose solution produced very little starch after five to seven days, while one of 8 per cent. produced abundance of starch in the above leaves.

My experiments in this direction have been conducted in a similar way to those of the investigators quoted. The sugars employed were cane-sugar, glucose, fructose, and maltose, varying from 5 per cent. to 20 per cent. solutions in water. The leaves used were cut into small fragments of 1 to 2 centims. in length, and carefully laid on the surfaces of the solutions, so as to remain floating, and thus have easy access to the oxygen of the air. Fragments that sank to the bottom of the dish were found to be free of starch, probably owing to lack of aeration. The glass dishes were covered with perforated glass plates, the holes being plugged with cotton wool. Although the solutions were boiled and allowed to cool before the pieces were floated, fungoid growths always made their appearance after the lapse of five or six days, their spores evidently being introduced on the surface of the leaves. Thus the experiments were only left as long as the mould was not present in any considerable quantity. The vessels were kept in a dark cupboard in the laboratory at the ordinary temperature, ranging between 15° and 17°. The leaf fragments were examined for starch, both macro- and micro-scopically. Care was taken to ensure that the material did not previously contain starch by the examination of the corresponding pieces. Having no invert-sugar prepared from cane-sugar at hand, the invert-sugar mentioned implies a mixture of equal quantities of glucose and fructose, which naturally acts similarly.

The following are the accounts of the experiments made with eight different species :—

Scilla sibirica. The leaves were taken from plants at the end of their flowering period. The solutions were 5 per cent., 10 per cent., and 20 per cent. cane- and invert-sugars. Some of the pieces examined after three days showed no starch.

Cane-sugar, after five days, 5 per cent.; starch in the cells round the bundles.

" " " 10 " ; more starch, but less than with 20 per cent.

" " " 20 " ; pieces put in iodine solution turned almost black,
hence large amount of starch.

The pieces in the invert-sugar solutions had much less starch, most in the 10 per cent.

Scilla nutans.

Exp. 1.—Leaves taken from plants on March 6. Solutions employed, 10 per cent., 20 per cent., and 30 per cent. cane-sugar, 10 per cent. glucose, 10 per cent. fructose, 10 per cent. invert-sugar, 10 per cent. and 20 per cent. maltose. In none of the cultures examined after four and six days did any of the leaf fragments show any starch in the mesophyll; only the guard-cells

* SAPOSCHNIKOFF, 'Ber. Deutsch. Bot. Gesell.,' 1891.

possessed starch, and this, of course, was present before. The 30 per cent. cane-sugar solution seemed to have plasmolysed many of the cells, and hence was too strong.

Exp. 2.—Commenced on May 12. Leaves of plants in flower. Used 10 per cent. cane-sugar, glucose, fructose, and maltose. No starch found at all in the mesophyll of any of the pieces examined after four, six, and ten days.

Exp. 3.—The upper parts of the leaves were picked on June 25, and placed on 10 per cent. and 20 per cent. cane-sugar solutions. The cultures were kept at a temperature of about 10°, and left for ten days before being examined. Nearly all the fragments remained starch-free, except the guard-cells; one or two pieces seemed to show a trace of starch.

Allium Cepa. Used the upper parts of the leaves of seedlings, presumably of the first foliage leaf after the cotyledon, as well as of the second one. Pieces floated on a 2 per cent. solution of cane-sugar, and examined after 6 days showed no starch at all, not even in the guard-cells of the stomata.

Asphodelus luteus. The upper parts of leaves from a plant not yet in bloom (May 15) on 10 per cent. and 15 per cent. cane-sugar and 10 per cent. glucose, fructose, and maltose solutions, and examined after five and seven days showed no starch, except in the guard-cells.

Hemerocallis flava. The experiment was carried out at the same time and under the same conditions as that with *Asphodelus*. The leaves were taken from a plant some time before blooming.

In 10 per cent. cane-sugar, a little starch, especially near the bundles.

„ 15 „ „ , a fair amount of starch.

„ 10 „ glucose, ditto.

„ 10 „ fructose, starch present, but less than in glucose.

„ 10 „ maltose, no starch.

Galanthus nivalis.

Exp. 1.—Leaves taken from plants well advanced in flower on March 6, and floated on 10 per cent. and 20 per cent. cane-sugar, 10 per cent. and 20 per cent. maltose, 10 per cent. invert-sugar, glucose, and fructose, when examined after four and six days showed no starch in any of the pieces.

Exp. 2.—The upper parts of the leaves were picked from plants not quite in flower on January 25, and submitted to 10 per cent. and 20 per cent. cane-sugar solution at a temperature of about 10°; they were examined after ten and twelve days. In most of the pieces no starch was visible, in a few a trace near some of the bundles, and the guard-cells had a little.

Narcissus poeticus (var. *ornatus*).

Exp. 1.—Leaves of plants in full bloom, February 22, on 5 per cent., 10 per cent., and 20 per cent. cane-sugar, and similar solutions of invert-sugar, showed no starch after three days.

After five days, on 5 per cent. cane-sugar, starch around the bundles and in the spongy mesophyll, but not in the palissade-cells.

„ 20 per cent. cane-sugar, starch in all the mesophyll-cells, but more in the spongy than in the palissade-cells.

„ 10 per cent. cane-sugar, about the same as 20 per cent.

The pieces floated on the invert-sugar solutions formed considerably less starch.

Exp. 2.—Leaves of plants which had finished blooming, on 10 per cent. solutions of cane-sugar, glucose, fructose, and maltose, and examined after four and six days, showed the following results:—

Cane-sugar, some starch in the cells round the bundles, and a little occasionally elsewhere.

Glucose, a minute trace of starch round some of the bundles.

Fructose, mere traces in some pieces, none in others.

Maltose, none.

Exp. 3.—Used leaves of a plant about 6 inches above the soil, the flower-buds being barely visible,

and of another just commencing to bloom. The solutions employed were 10 per cent. and 20 per cent. cane-sugar, 10 per cent. and 20 per cent. maltose, 10 per cent. glucose, fructose, and moist sugar; examined the pieces after four and six days. To distinguish the leaves of the two ages, " α " signifies the younger, and " β " the older ones of the flowering plant.

Cane-sugar, 10 per cent., starch plentiful in both, least in the palissade layers, and probably more in α than β .

„ 20 per cent., on the whole less starch than in 10 per cent.

Invert-sugar, 10 per cent., α , fair amount round the bundles, not much elsewhere.

„ „ „ β , starch in some of the bundle cells, but less than in α .

Glucose, 10 per cent., α , starch throughout the mesophyll, except the palissade layers.

„ „ „ β , not as widely distributed as in α .

Fructose, 10 per cent., α , a little round the bundles, but not as much as in the 10 per cent. glucose.

„ „ „ β , none visible microscopically.

Maltose, 10 per cent., α , starch in one or two cells near the bundles in some pieces.

„ „ „ β , as in α , but not so apparent. Starch in both invisible microscopically.

„ 20 „ similar to 10 per cent., but less in amount.

Exp. 4.—From a plant which had just finished flowering. Used 10 per cent. and 15 per cent. cane-sugar, 10 per cent. glucose, fructose, and maltose solutions, and examined after five and seven days.

Cane-sugar, 10 per cent., small amount of starch round the bundles, extending some way in the spongy tissue.

„ 15 „ less in amount than in 10 per cent.

Glucose, mere trace in some of the phloëm cells.

Fructose, a little round the bundles.

Maltose, none.

Iris flavescens.

Exp. 1.—Leaves taken from plants just showing their flower-buds, in 10 per cent. and 15 per cent. cane-sugar, 10 per cent. glucose, fructose and maltose, and examined after five and seven days.

Cane-sugar, 10 per cent., plenty of starch: blackish in patches after iodine.

„ 15 „ fair amount, but less than in 10 per cent.

Glucose, similar to 15 per cent. cane.

Fructose, ditto.

Maltose, none.

Exp. 2.—Leaves of plants just commencing to flower, in 10 per cent. solutions of cane-sugar, glucose, fructose, and maltose.

Cane-sugar, plenty of starch in the mesophyll.

Glucose, less starch.

Fructose, similar to glucose; perhaps rather more.

Maltose, none in the mesophyll.

Conclusions.

(1.) Of the five sugars used, cane-sugar was pre-eminently the best starch-producer, thus confirming previous workers. As regards the strength of the solution, in the case of *Narcissus* and *Iris*, 10 per cent. is as advantageous as, or more so than, 15 per cent. or 20 per cent., while *Scilla sibirica* seemed to form more starch from a 20 per

cent. than a 10 per cent., and *Hemerocallis* more from a 15 per cent. than a 10 per cent. solution. On the whole the optimum concentration comes somewhere near that of SAPOSCHNIKOFF's for *Vitis* and *Rubus*, viz., 10 per cent.

(2.) With respect to the other sugars, there is not much difference between glucose and fructose, any advantage being usually in favour of the former. Invert-sugar comes about the same, while maltose is generally unfavourable.

(3.) The leaf-pieces have to float at least four days on the solutions before starch makes its appearance.

(4.) The starch is deposited centrifugally from the vascular bundles, gradually extending outwards from these, and appearing last of all in the outer or palisade layer of cells.

(5.) Considering the individual behaviour of the different leaves, *Iris* and *Scilla sibirica* formed starch in large quantities, *Narcissus* in moderate amount, *Hemerocallis* only a little, while *Scilla nutans*, *Allium Cepa*, *Asphodelus* and *Galanthus* failed to form any. The onion leaf has been shown by BÖHM,* as well as by RENDLE†, to be incapable of producing starch from sugar-solutions. BÖHM also failed with *Asphodelus*, but was successful with *Galanthus*. The case of *Scilla nutans* is rather strange in differing so much from that of its fellow species, *S. sibirica*; but it is significant that *S. sibirica* stores starch plentifully in its bulb, while *S. nutans* does not. Thus there is a parallelism between the behaviour of the leaf and that of the reserve organ. This is also seen in *Allium Cepa* and *Asphodelus luteus*, which have reserve organs destitute of starch. However, *Hemerocallis* is an exception on the one hand, and *Galanthus* on the other. *Narcissus poeticus*, as the experiments show, can form starch in its mesophyll at various periods of its growth, but more when the leaves are young.

(6.) Since invert-sugar is not so productive of starch as cane-sugar, it seems that the latter is converted to starch without being first inverted. BÖHM thought the contrary, finding a considerable amount of invert-sugar in the solutions after his experiments, but this might be due to the invertive action of moulds. MEYER, however, gave strong proof that the cane-sugar is not first inverted into glucose and fructose.

PART II.—THE OCCURRENCE OF STARCH AND INULIN IN THE RESERVE ORGANS OF MONOCOTYLEDONS.

Under this heading are given the results of some analytical work and microscopic observations on the organs for storing reserves of food possessed by several Monocotyledons. I propose to give:

(I.) An account of the inulins isolated from the bulbs of *Scilla nutans* and *Galanthus nivalis*.

* BÖHM, *loc. cit.*

† RENDLE, *loc. cit.*

- (II.) The results of the applications of microchemical tests for inulin to a number of monocotyledonous reserve organs.
- (III.) Microscopical observations on the starch and inulin in the bulb of *Galanthus nivalis* during its annual growth, and a comparison of it with the bulb of *Narcissus Pseudo-Narcissus*.

Historical.

A. MEYER* gives conclusive evidence of the presence of an inulin in the rhizome of *Yucca filamentosa*, as well as in its leaf. As the substance isolated appeared identical in properties with SCHMIEDEBERG's Sinistrin, found in the bulb of *Scilla maritima*, he gave it that name.

In TOLLEN's 'Handbuch der Kohlenhydrate' several compounds are named in the section of the book devoted to inulin and its allies, which produce fructose (levulose) on hydrolysis, and which are obtained from monocotyledonous reserve organs. The following is a list of them :—

Sinistrin	from	<i>Scilla maritima</i> .
Triticin	„	<i>Triticum repens</i> .
Phlein	„	<i>Phleum pratense</i> and <i>Phalaris arundinacea</i> .
Irisin	„	<i>Iris pseudacorus</i> .
Graminin	„	<i>Trisetum alpestre</i> , <i>Agrostis</i> , <i>Festuca</i> , &c.

These have been isolated by various chemists, analysed, and their properties and specific rotatory powers ascertained, and shown to yield fructose on hydrolysis. TOLLEN considers them all identical. They are all levorotatory, differing a little in the amount of rotation from ordinary inulin (*i.e.*, of Compositæ) and between themselves. They all yield fructose on treatment with dilute acids, are very difficult to obtain crystalline, are usually isolated as gums, and are generally fairly soluble in cold water, differing in these last two properties from ordinary inulin.

Another communication on the inulins of Monocotyledons has recently been contributed by CHEVASTELON, in the form of a thesis for D.Sc. at Paris in 1894. I have not been able to secure the original, but have perused a summary of it in the 'Journal de Pharmacie.' The paper is concerned with the inulin of Garlic, Hyacinth, Asphodel, and Tuberose. He first gives the mode of preparation, and then the properties of the substances isolated. The body from garlic is soluble in all proportions in cold water, soluble in 70 per cent. alcohol, but only slightly so in 95 per cent. and absolute alcohol; thus, he says, it cannot be made visible by the microscope. It is turned to fructose by mineral and organic acids. The inulin in the Hyacinth is very similar to that of the Garlic. The inulins of Asphodel and Tuberose are also similar.

* MEYER, 'Bot. Zeit.,' 1885.

SECTION 6.—THE INULINS OF *Scilla nutans* AND *Galanthus nivalis*.

The word inulin is here used in an extended sense to embrace all those carbohydrates which produce fructose only, or, at any rate, mainly, on hydrolysis with an acid. Consequently, the only sure test of a substance being of the nature of an inulin is to isolate and purify a sufficient quantity of it, so as to be able to ascertain its specific rotation both before and after hydrolysis. This has been done in the case of two plants.

Scilla nutans.—The bulb contains no reserve starch. I have examined it several times at various periods of the year, and have never found any starch in the bulb-scales; it occurs only in small amounts towards the apex of the axis of the bulb. The bulb-scales are tunicate and somewhat fused together at the base, consisting only of foliage leaf-bases, and lasting one year as reserve organs. The tissue of the scales is somewhat translucent and very sticky, due to the mucilage of the numerous raphide-cells. To extract inulin the following method was pursued:—

The fresh bulbs were mashed up, and the pulp boiled with water and then filtered. To the filtrate a large excess of 95 per cent. alcohol was added, which brought down a copious precipitate, which easily dissolved again in water. Alcohol was added a second time to the solution by degrees, when first a precipitate of gelatinous flocks separated out; this is the mucilage of the raphide-cells, which is precipitated by a lower percentage of alcohol than the inulin. After its removal more spirit is added, which brings down the inulin as a white finely-divided precipitate. The operation is repeated a few times in order to remove the mucilage as much as possible from the inulin, which is finally dried on a porous plate and obtained as a white powder. About 3 grams of this were got from two dozen large bulbs. The following experiments were carried out with this material so prepared, in order to test its inulin nature.

1.396 grams of the powder, dried at 100° and dissolved in water, gave an angle of rotation in the 200 millims. tube of $-1^{\circ}06$. This corresponds to a specific rotation of $[\alpha]_D - 37.9$. Inulin from *Helianthus tuberosus* has a specific rotatory power of $[\alpha]_D - 40^{\circ}$.

50 cub. centims. of the original solution of the inulin from *Scilla nutans* was then hydrolysed with .5 per cent. oxalic acid. After removal of the oxalic acid with calcium carbonate, and making up to 100 cub. centims. with water, the polarimetric reading in the 200 millims. tube was -1.13 , therefore the angle of rotation on the original solution was $-2^{\circ}26$, corresponding to 1.244 grams of fructose. Further heating of the solution with acid proved that the hydrolysis had been complete.

The hydrolysed solution, on boiling with Fehling's solution, under the conditions laid down by BROWN, MORRIS, and MILLAR,* gave an amount of cupric reduction equivalent to 1.35 grams of fructose per 100 cub. centims., against 1.55 grams as calculated from the amount of inulin originally present. This small discrepancy is probably due to a certain amount of destruction of the fructose during the process of hydrolysis.

Thus the inulin-nature of this carbohydrate, prepared from the bulb of *Scilla nutans*, appears fairly well established, since the numbers obtained for the opticity and cupric reduction after hydrolysis agree closely with those required on the

* 'Journ. Chem. Soc.,' 1897, p. 280.

assumption that fructose is the sole hydrolytic product. The lævorotatory power of the carbohydrate also corresponds very closely with that of ordinary inulin.

The characteristic property of this inulin is its easy solubility in cold water, thus differing from the inulin of the Compositæ, which requires a temperature of about 50° before any considerable amount goes into solution. Thus it agrees with the inulins prepared by CHEVASTELON* from *Hyacinthus*, &c. Like the latter, it also needs a high percentage of alcohol to precipitate it.

Galanthus nivalis.—The bulb contains starch, as well as the carbohydrate of which the inulin nature is now to be shown. The compound was prepared in the following manner, from the dried bulb scales ground to a powder. The material was boiled up with water in order to thoroughly gelatinise the starch. After cooling, some active malt extract was added to convert the starch completely into maltose and dextrin. Then the decoction was boiled again to bring the inulin wholly into solution, and filtered hot. The filtrate contains the inulin, dextrin, sugars, and the mucilage of the raphide-cells, which, however, are not nearly so abundant as in *Scilla nutans*. On allowing the liquid to stand for some 24 hours, the inulin gradually separates out and falls as a white precipitate to the bottom of the vessel. The separation can be accelerated by surrounding the vessel with ice. The supernatant liquid is then poured off, thus removing most of the dextrin, sugars, and mucilage. The inulin left is boiled up with distilled water, and again allowed to separate out. A few repetitions of this operation effectually remove the dextrin and mucilage. The inulin is then further purified by precipitating it from its solution in water by alcohol. Any sugar still remaining, being soluble in the spirit, is washed away. The compound is in this way obtained as a whitish powder. It is necessary to convert the starch, because it is gelatinised by the boiling, and cannot as such be well separated from the inulin. Owing to the inulin coming out of solution on standing, its isolation is insured from the dextrin and mucilage, which, if not removed in this way, would remain with the inulin after the addition of the alcohol.

Experiment to test the inulin character of the substance :—

·365 gram of the dried compound was dissolved in boiling water; when the solution had cooled to the ordinary temperature (15° C.), readings were taken in the polarimeter.

(α) Angle of rotation in the 200 millims. tube = -3° .

A part of the solution was hydrolysed by a 1 per cent. solution of oxalic acid and the opticity again determined.

(β) Angle of rotation in the 200 millims. tube = -73° .

Calculating out the specific rotation of the original substance the following was obtained :—

(α) $D = -43.8^{\circ}$.

TOLLENS gives the specific rotation of ordinary inulin (*i.e.*, Compositæ) at 15° C. as about

(α) $D = -40^{\circ}$, thus the two values are fairly approximate.

The amount of fructose corresponding to the angle -73° is .4019 gram.

The theoretical quantity of fructose obtainable from .365 gram. of inulin ($C_6H_{10}O_5$) is .4055 gram.

* *Loc. cit.*

Solubility of the inulin. Since ordinary inulin dissolves readily in water at about 50°, it seemed feasible to attempt the quantitative estimation of the inulin apart from the starch in the snowdrop bulb, by dissolving out the former at a temperature of 50° to 55°, which would not be sufficiently high to gelatinise the starch. On attempting this I found that, whereas on the one hand the starch remained unaltered—the filtered liquid giving no blue coloration with iodine—on the other hand, no appreciable amount of inulin could be brought into solution. This led me to experiment on the solubility of the prepared sample, comparing it with ordinary inulin, using distilled water as the solvent.

- Experiments.*—(1.) Temperature, 50° to 55°. The ordinary inulin was dissolved after some hours, but none of the snowdrop inulin had dissolved after two days.
- (2.) Temperature, 61° to 62°. The ordinary inulin naturally dissolved readily, but the snowdrop inulin showed no sign of going into solution, although the starch grains swelled and the filtered liquid gave a blue colour with iodine.
- (3.) Temperature, 70° to 72°. The snowdrop inulin remained undissolved after six hours.
- (4.) Temperature, 76° to 77°. Still remained undissolved.
- (5.) Temperature, 80°. The inulin now began to dissolve. Hence, a temperature of 80° or over is required to bring the inulin of the snowdrop bulb readily into solution, whereas ordinary inulin dissolves easily at 55°, or even below. Consequently, the inulins prepared from the reserve organs of the plants, *Scilla nutans*, *Helianthus tuberosus*, and *Galanthus nivalis* present three degrees of solubility in water. The first is soluble in cold, the second in warm, and the third in hot water.

SECTION 7.—THE EXAMINATION OF RESERVE ORGANS OF MONOCOTYLEDONS FOR STARCH AND INULIN. (Plate 3.)

The presence of starch in a reserve organ is readily ascertained under the microscope, but not so that of inulin, because in the living tissue it is in solution in the cell sap; it can, however, be precipitated in the tissue by alcohol, and assumes the form of sphero-crystals, so well known in tubers of *Helianthus tuberosus* and *Dahlia variabilis*. Although the formation of the sphero-crystal is thus characteristic, in Monocotyledons the inulin appears not to assume the crystalline shape when precipitated in the tissues, but occurs as amorphous masses. Consequently it seemed desirable to search for some microchemical tests for inulin capable of application to sections of the alcoholic material.

In ZIMMERMANN'S 'Botanische Microtechnik' (Eng. transl., p. 76), the following test, by MOLISCH, is given for carbohydrates such as sugars and inulin:—Treat thick sections of the tissue with a drop of 10 per cent. to 20 per cent. alcoholic solution of α -naphthol, and then add to it two to three drops of concentrated sulphuric acid, when in a couple of minutes or less a beautiful violet coloration ensues. By experimenting with this test on pure carbohydrates, it seems really to afford a means of discriminating between fructose-yielding* carbohydrates and others. Fructose,

* Fructose and glucose are used in preference to the old terms levulose and dextrose.

saccharose (cane-sugar), and inulin give a deep violet colour at once, while glucose and maltose afford merely a pink coloration, and starch and cellulose do not react in any characteristic manner.

GREEN, in his researches on the germination of the Artichoke tuber ('Annals of Botany,' 1888), employed an alcoholic solution of orcin and hydrochloric acid as a test for the spherocrystals of inulin in sections of the tuber, steeping them first in the solution and then heating them in the acid; a deep orange stain is left in the space formerly occupied by a spherocrystal. I have experimented with a 10 per cent. solution of orcin and concentrated sulphuric acid, instead of hydrochloric acid, and here again find it a test for fructose. Employing pure material, and adding to a little of it on a slide a drop of the orcin solution and then a drop or two of sulphuric acid, fructose, saccharose, and inulin furnish an immediate deep orange colour, whereas glucose and maltose give only a slight brownish tint. These two tests, the α -naphthol and the orcin, are those employed in these investigations. It must be borne in mind, then, that the tests are not exclusively for inulin, but rather for fructose, and for those carbohydrates capable of producing this sugar on hydrolysis. Consequently carbohydrates, insoluble in strong alcohol, which yield fructose only, readily respond to those reagents, and such are denoted inulins; thus the tests come to be distinctive for inulin. It is quite possible that there may be in plants carbohydrates (polysaccharides) insoluble in alcohol, which give rise to another hexose ($C^6H^{12}O^6$) besides fructose; but, as such are at present unknown, bodies precipitated in the tissues by alcohol, and reactive to the tests, may with considerable certainty be taken as inulin, especially since, as is shown further on, the form which the precipitate takes in the cells is the same for *Scilla nutans* and *Galanthus nivalis* (the inulin nature of which has been proved chemically in Section 6) as for the other plants. Of course it is necessary to be careful that no sugars are present in the sections to be tested, since these might react, and thus the presence of inulin be inferred when it is not present. To avoid this, the sections are soaked in alcohol for some time to dissolve out any sugars before applying the tests. When the colorations ensue, there is usually confirmatory evidence of an inulin substance from the appearance of the cells in the spirit material. They often have a distinct precipitation in them, either in the form of spherules (fig. 5), when sections of the fresh tissue are put in alcohol, or as a thick lining (sometimes completely filling the cell-cavity, fig. 10) to the interior of the cell-wall (figs. 1, 7, 8a, 9) when sections are cut from the reserve organ, thoroughly soaked in spirit. On placing the sections in cold water this matter in the majority of cases dissolves (fig. 8b), and now the colorations no longer appear, thus proving that they are caused by this amorphous substance. The exceptions occur in the bulbs of *Galanthus* and *Leucojum*; here the precipitate remains insoluble in cold water, and the sections thus treated continue to respond to the tests.

It will be well to commence the descriptions of the microscopic appearance of the

starch and inulin in the reserve organs with those of *Scilla nutans* and *Galanthus nivalis*, because their inulins have been investigated chemically in Section 6, and may thus serve as two types to which the others may be referred.

Scilla nutans. I have examined the bulb several times at various periods of the year, and never found any starch in the bulb-scales; it occurs only in small amounts towards the apex of the axis of the bulb in minute granules. Sections of the bulb preserved for some time in alcohol do not show any spherocrystals, but the parenchymatous cells have amorphous deposits (fig. 1). The alcohol sections give pronounced colorations with the α -naphthol and orcin tests. A drop of the naphthol solution is put on the section, and then a couple of drops of sulphuric acid added, when almost immediately a deep violet colour spreads all over it. With orcin the coloration appears in a similar way as a deep orange-brown. After steeping the sections in cold water, they no longer respond to the tests, and the precipitate has disappeared from the cells; this agrees with the fact that the prepared inulin from the bulb is soluble in cold water. Thus the evidence that the substance deposited in the cells by the spirit is the inulin is fairly conclusive. The abundant mucilage of the numerous raphide-cells may interfere with a clear view of the behaviour of the inulin, since in water it does not at once dissolve, but swells up, and is apt to obliterate the view of the parenchymatous cells. It has, however, no connection with the tests, because a section, after being in water, does not react with the reagents, although it may have a lot of mucilage still adhering to it. Some of the reserve organs devoid of, or with much fewer raphide-cells, to be described later, show the appearance and disappearance of the inulin matter much better. The stored carbohydrate material in the bulb of this plant then is largely, most likely solely, an inulin, reserve-starch being entirely absent.

Galanthus nivalis. The bulb-scales contain a considerable amount of starch (45μ).^{*} On examining bulbs soaked for some time in alcohol, the starch grains in each parenchymatous cell are seen to be embedded in a solid matrix filling up the entire cell-cavity (fig. 2). Sections of the scales give very marked reactions with the α -naphthol and orcin tests. On steeping them in cold water and then applying the reagents, the colorations result as before, and the embedding matter is seen to be undissolved. Sections of fresh bulb-scales show the cells quite clear, except for the starch grains (fig. 3). When such a section is placed in alcohol, spherules make their appearance in the uninjured parenchymatous cells (fig. 5), and do not dissolve in cold water. Such cells are coloured in the characteristic manner by the inulin tests. The inulin which escapes from the injured cells is precipitated by the alcohol as minute granules. Portions of fresh bulb-scales, put into spirit and left for a day, and then examined, showed the inulin deposited throughout the parenchyma; in the outer cells it had the form of spherules, and gradually, as the superficial sections

* The numbers in brackets indicate the length of the largest starch grains in the various reserve organs containing this carbohydrate.

were removed, it was seen to assume the characteristic form of casts of the cells. It thus appears that the form taken by the inulin, when precipitated, depends on the rapidity of penetration of the alcohol. Moreover, it is possible that the spherules in a cell coalesce to form one mass. The inulin casts are easily freed from the cell-walls enclosing them, and float about independently with the starch grains embedded in them (fig. 4). That the casts are in the main really inulin, follows from the fact that both the sections of the spirit material and the prepared snowdrop inulin respond to the tests, and also from the fact that the solubility of the casts pursues the same course as that of the prepared substance (see Section 6).

In distilled water at a temperature of about 62° the casts in the sections remain intact, although the starch grains in them swell, and the liquid is rendered blue with iodine solution. They do not begin to dissolve till a temperature of about 80° is reached. However, in a 0·5 per cent. hydrochloric acid solution at 62°, the casts dissolve slowly, as does the prepared inulin, and when they have disappeared the sections cease to give the test-colorations. The starch grains are easily dissolved out of the sections by saliva at a temperature of about 30°, leaving the casts intact, with cavities in them, formerly occupied by the grains (fig. 6). When examined by polarised light, the casts do not suggest crystalline structure and are most likely amorphous. Further details respecting the starch and inulin are given in Section 8.

The special points to notice here are that the bulb-scales of *Galanthus nivalis* store starch and inulin, probably in about equal quantities, that the inulin is insoluble in cold or even in warm water, and that it is precipitated by alcohol in the cells in a characteristic manner.

In the following *résumé*, the plants are taken in their systematic order and their reserves described in a concise manner.

HYACINTHEÆ.

Scilla sibirica. The scales of the bulb contain a moderate amount of starch (15 μ). Sections preserved in alcohol give at once the violet coloration with the α -naphthol reagent, and the orange with the orcin; after steeping them in cold water the tests do not react, thus showing that the matter which reacts has been removed by the water. It is interesting to note that two plants of the same genus differ markedly in their reserve carbohydrates; while *S. nutans* stores only inulin, *S. sibirica* stores starch as well.

Ornithogalum byzantinum. The bulb possesses plenty of starch. The inulin reactions are clear, but not after soaking the sections in cold water.

Hyacinthus orientalis. The bulb-scales contain plenty of starch (25 μ). Thick sections of the fresh scale when put in alcohol show a granular precipitate in the parenchymatous cells and give the inulin reactions; after treatment with water the granularity disappears and the reactions do not take place, thus the deposit appears to be the inulin separated out by the spirit. A. MEYER, in his treatise on Starch,* devotes a chapter to his investigations on the deposition and dissolution of the starch grains in the Hyacinth bulb, but does not mention the occurrence of inulin in it. CHEVASTELON found inulin in it (see Section 6).

* 'Unters. ü. d. Stärkekörner,' 1895, p. 294.

Muscari botryoides. The bulb has numerous scales with plenty of starch (33μ), and gives the inulin reactions with the same appearance in alcohol as the Hyacinth.

Chionodoxa Lucilae. The bulb is similar to that of *Muscari*, and gives the reactions for inulin.

Allium Cepa. RENDLE ('Annals Bot.,' II., 1889) says that starch is usually present in small quantities in the parenchymatous cells round the vascular bundles in the succulent scales of all ages, as well as in the general parenchyma of the bulb's axis. I have found starch in the axial region of the bulbs at various stages of growth, as well as in *A. Porrum*, but none in the scales of the older bulbs. Sections of a scale in alcohol show a granular precipitate and give the characteristic reactions for inulin, but not after water, which clears them.

Allium sativum. Thick sections of the scales of fresh resting bulbs were quite clear when mounted in water; when put in alcohol a deposit was formed round the inside of the cell-walls, which dissolves on placing again in water. Sections in alcohol give the α -naphthol and orcin reactions. No starch found anywhere in the bulb, not even in the axis.

Allium Schœnoprasmum. A little starch is contained in the layer of parenchymatous cells around the vascular bundles of the scales. A granular precipitate is formed in the cells by alcohol, and sections give the inulin reactions. The granules are dissolved by cold water, and the tests do not then succeed.

Triteleia uniflora. The scales of the bulb have plenty of starch (16μ). Inulin occurs as in *Allium* (fig. 7).

ANTHERICEÆ.

Anthericum liliago. Plant taken up for examination on June 15th, when in full leaf. The reserve organs consist of fleshy roots, devoid of starch. The cells of the fresh tissue are quite clear, but after alcohol a dense granular precipitate is seen filling the cells: it dissolves again in water. The alcoholic sections give the inulin reactions forthwith, but not after soaking in water.

Asphodelus luteus. Plant taken up on June 15th, just at the end of its flowering period. The reserve organs consist of thick fleshy roots, the old ones being almost shrivelled up. Sections of the fresh root revealed no starch, but the tissues behaved in alcohol and to the tests as did *Anthericum*.

Yucca filamentosa. Fresh rhizome examined on June 1st. No starch except in a layer of cells between the cortex and stele. The parenchymatous cells, quite clear in the fresh tissue, when placed in alcohol become cloudy, and the uninjured ones are seen to be full of spherules (fig. 8), and when placed in water these are immediately dissolved. The alcoholic sections respond to both tests, but not after being in water.

Funkia ovata. The short knotty rhizome contains no starch. In the part of the rootstock just below the terminal bud some starch in very small granules occurs. Material in alcohol shows a deposit in the cells, soluble in water and reactive to the tests.

Hemerocallis flava. The reserve organs consist of tuberous roots, containing no starch and reacting as in *Funkia*. A little starch occurs in the bud regions.

H. fulva is similar to *H. flava* as regards inulin and starch.

Tritoma uvaria. The roots are thick, but not tuberous. They possess no starch, but react to inulin like *Hemerocallis*.

Convallaria majalis. A moderate quantity of starch in the rhizome and roots: small grains (7μ). Sections in alcohol give the inulin reactions at once, but not after being in water.

Polygonatum multiflorum. The Iris-like rhizome contains no starch. The spirit material exhibits the parenchymatous cells with a mucilaginous matter which dissolves at once in water and is evidently inulin (fig. 9), for the sections give the reactions, but not after they have been cleared by water. Starch only occurs in the region of the terminal bud.

Asparagus officinalis. The roots function chiefly as the storage organs and contain no starch, but give to some extent the inulin reactions.

Ruscus aculeatus. Some of the rootstock taken up on July 7th. No starch found, but the cells of the roots after treatment with alcohol were full of granular matter and gave the reaction for inulin.

AMARYLLIDACEÆ.

Galanthus. Bulbs of *G. Imperati*, *G. Elwesii*, *G. latifolius*, and *G. plicatus* were examined and found to contain starch and the same kind of inulin as *G. nivalis*, previously described.

Leucojum vernum and *L. æstivum*. The bulbs of these two plants resemble those of *Galanthus* in the form of their starch-grains and in the character of their inulin, which assumes the form of casts, and is insoluble in cold water. Chemical proof of the presence of an inulin in the bulb of *Leucojum vernum* has been given by EHRHARDT.* From the fact that the substance he isolated from the bulb was with difficulty soluble in cold water and had a specific rotatory power near that of ordinary inulin, he considered it to resemble more closely the latter than such bodies as sinistrin, tritacin, &c., hitherto obtained from Monocotyledons. He, however, was unable to procure it in the form of sphero-crystals. The above agrees with the properties of the inulin which I have prepared from the bulb of *Galanthus nivalis*, and also with the nature of the inulin casts precipitated in the cells by alcohol, which are insoluble in cold water and not crystalline in structure.

Polygonum tuberosum. The tuber possesses no appreciable amount of starch—a few very small grains occur in the cells between the cortex and stele. Inulin is presumably present, since alcohol produces in the cells a copious precipitate, again soluble in cold water and reactive to the tests.

IRIDACEÆ.

Iris pseudacorus. The rhizome has only a little starch and the tests for inulin are not conclusive.

I. plicata. Plenty of starch in small grains in the rhizome. Sections in alcohol respond moderately to the inulin tests, but not after water.

I. orchidoides. The bulbous rootstock is full of starch (20μ). Fresh sections put in alcohol give a granular precipitate, soluble again in cold water. Good colorisations with α -naphthol and orcin. Absent after treatment with water. The thick roots possess starch and also give the reactions.

I. Xiphium has a bulbous rootstock, exhibiting the same features as *I. orchidoides*.

GRAMINEÆ.

Phleum pratense. The base of the stem swells into a tuber embracing two or three internodes. No starch occurs in the tuber or in the narrow rhizome. Sections of the tissue preserved in alcohol are full of granular matter, which is slowly dissolved by cold water, not stained by iodine, and at once gives marked reactions with α -naphthol and orcin (fig. 10).

ARACEÆ.

Arum maculatum. The rootstock consists of a tuberous rhizome, containing abundance of starch in small angular grains, often compound (8μ). Evidence of inulin not conclusive.

The following plants were found to contain starch, but no inulin, in their reservoirs of reserve-materials:—*Uvularia grandiflora*, *Veratrum nigrum*, *Colchicum autumnale*, *Tulipa* (sp.), *Fritillaria Meleagris*, *F. imperialis*, *Erythronium Dens-canis*, *Lilium tigrinum*, *L. Martagon*, *Crocus vernus*, *C. aureus*, *Sparaxis* (sp.), *Babiana* (sp.), *Schizostylis coccinea*, *Gladiolus cardinalis*, *Freesia refracta*, *Listera ovata*, *Orchis maculata*, *O. mascula*, *Tradescantia virginica*, *Sparganium ramosum*, and *Alisma Plantago*.

In *Crinum podophyllum* the presence of inulin is doubtful, and still more so in *Sternbergia lutea*, *Amaryllis belladonna*, *Eucharis amazonica*, and *Pancratium speciosum*, all of which are probably devoid of it.

* EHRHARDT, 'Bot. Centralbl.', 1894, vol. 60, p. 207.

Summary and General Remarks.

The Monocotyledons here dealt with can be divided into four categories according to the nature of their reserve carbohydrates, viz. :—

(1.) Those which contain starch alone, include the Colchicaceæ, Tulipeæ, *Narcissus*, *Alstræmeria*, Ixiæ, Gladiolæ, Orchidaceæ, *Tradescantia*, *Arum*, *Sparganium* and *Alisma*.

(2.) Those which contain inulin alone, soluble in cold water, include *Scilla nutans*, *Allium*, Anthericæ, *Yucca*, Hemerocallidæ, *Polygonatum*, Asparagæ, *Polianthes* and *Phleum*.

(3.) Those which contain both starch, and inulin soluble in cold water, include the Hyacintheæ (except *Scilla nutans*), *Triteleia*, *Convallaria* and *Iris*.

(4.) Those which contain both starch, and inulin insoluble in cold water, include *Galanthus* and *Leucojum*.

The starch-grains are usually characteristic for whole groups. The Colchicaceæ possess fairly small, often compound grains, the Tulipeæ large eccentric grains, the Hyacintheæ fair-sized ones, the Amaryllidæ large spherical ones, the Iridaceæ as a rule small compound grains, the Orchidaceæ cylindrical ones of considerable size.

Although the evidence for the occurrence of inulin is chiefly microscopic, yet, comparison with those plants in which the true nature of the carbohydrate has been determined chemically, shows it to be fairly conclusive.

Besides inulin like that of the Compositæ, substances with similar properties appear to have a wide distribution in Monocotyledons. Fructose-yielding compounds may be, next to starch, the most generally distributed reserve-carbohydrates. Contrary to what has often been inferred, starch and inulin can occur together in the same reserve-organ, as is well seen in the bulb of *Galanthus*. In all the cases investigated the inulin exists in solution in the cell-sap and is precipitated by alcohol, and except in *Galanthus* and *Leucojum* it is soluble again in cold water. The inulin of *Helianthus tuberosus* when thus precipitated is insoluble in cold water, and so resembles that of *Galanthus*; but the latter is less soluble in warm water. It is therefore quite likely that there is a series of fructose-yielding carbohydrates diminishing in molecular complexity and increasing in solubility, as in the case of the starch series.

The two species of *Scilla* are interesting, because here are two plants of the same genus, one (*S. sibirica*) containing starch as well as inulin in its bulb, and the other (*S. nutans*) storing only inulin. A further examination of the genus would be instructive, to see what other species also lack starch in the bulb, and might shed some light on the question why one reserve-organ should store starch, another inulin, and a third both of these carbohydrates together. The reason, at present, seems obscure, and it is somewhat hazardous to attempt to draw conclusions from the relatively few monocotyledonous reserve-organs examined. However, from these

investigations the following suggestions are here offered, as having perhaps something to do with the solution of the problem.

The reserve-organs of aquatic plants, and of plants inhabiting damp situations, seem not to possess inulin, but only starch, *e.g.*, those of *Sparganium*, *Alisma*, *Listera*, *Orchis*, *Schizostylis*; whereas the plants storing inulin often grow in dry and sandy soils, notable examples being *Allium*, *Asphodelus*, *Anthericum*, *Yucca*, *Tritoma*, *Asparagus*, *Iris orchidoides* and *I. Xiphium*. Since inulin exists in solution in the cell-sap, a storage-organ containing it may be better able to resist desiccation than one full of starch. The effect of the inulin dissolved in the sap would retard the evaporation of the water from the parenchymatous tissue.

There seems to be a certain correspondence between the absence of starch in the foliage leaves (or rather their incapacity for forming starch) and its absence from the reserve-organs, and *vice versa*. This is strikingly exemplified in the case of *Scilla*. Portions of the leaves of *S. sibirica* produce starch copiously when floated on a solution of saccharose, whereas those of *S. nutans* do not; the former contains starch in its bulb, the latter not. (See Part I. on Starch in Monocotyledonous Leaves.)

The nature of the carbohydrate of the reserve-organ may depend largely on the kind of sugar passing out of the leaf to the storage-organs; if it be chiefly saccharose, then starch might be expected to be elaborated; if fructose, inulin.

SECTION 8.—MICROSCOPICAL OBSERVATIONS ON THE STARCH AND INULIN IN THE BULB OF *Galanthus nivalis* DURING ITS ANNUAL GROWTH, AND COMPARISON WITH THE BULB OF *Narcissus Pseudo-Narcissus*. (Plate 4.)

A. *Galanthus nivalis*, LINN.

Before commencing the description of the special work, it may be well to give a brief account of the general structure of the bulb.

Morphology of the Bulb.—The remarks to be made result from observations on a number of bulbs, dug up during September, 1896, in a garden where they had been growing in clumps undisturbed for some years.

Each plant possesses two foliage leaves, or, if the bulb has been newly formed, perhaps only one, but in no case more than two. This number seems constant for the species, whereas in the case of *Narcissus Pseudo-Narcissus*, the leaves vary in number from two to four.

The bulb has three fleshy scales, two of these being the sheaths of the dead foliage leaves, and the other the lower part of a sheathing phyllome structure, external to the foliage leaves, which is termed the tunic. Thus, commencing from the exterior of the bulb, there are, first the old exhausted brown scales, then the base of the tunic, forming a tunicate scale, then the sheath of the outer foliage leaf, also a tunicate

scale, and after this the base of the inner foliage leaf, which is a partial scale enclosing the lower part of the floral scape, this also storing nutriment; in the centre is the bud, which will expand into tunic, foliage leaves, and flower the next year, while their bases will accumulate reserve material during the assimilating period to form fresh succulent scales, and so on (figs. 1 and 2). The bulb-scales last only one year as reserve organs, and not, as in the case of *Narcissus*, for a longer period. The buds, which give rise to new bulbs, usually arise in the axils of the tunics, one to each.

Such are the general external features of a bulb, gathered from the examination of nearly a thousand of them. Other details were noticed, such as the presence in a few bulbs of an extra scale, suggestive of the vestige of a second tunic, also an occasional one with the axis considerably lengthened between the scales and the new bud, in fact, drop-bulbs.

Histology of the Bulb.—Transverse and longitudinal sections of a bulb-scale reveal the following characters (see fig. 4):—

(1.) Two epidermal layers composed of small cells, elongated in a longitudinal direction. The one limiting the scale on the outer side represents really the morphological lower surface of the leaf, and the other on the inner surface the upper surface of the leaf. They are referred to respectively as the outer and inner epidermis.

(2.) A mass of storing (parenchymatous) tissue, enclosed between the two epidermal layers.

(3.) The fibro-vascular bundles running for the most part longitudinally, small in sectional area, and consisting of a few vessels (xylem), which naturally face the inner epidermis, and a little phloem, next the outer epidermis.

(4.) Raphide-cells, arranged in vertical rows, and more numerous in the outer half of the scale, below the outer epidermis. These cells are much elongated longitudinally, contain each a bundle of raphides, about $65\ \mu$ in length, and enclosed in mucilage.

The storing tissue, to which attention is more specially directed, consists of ordinary parenchymatous cells, polygonal in outline, with thin cellulose walls, and on an average about $100\ \mu$ in diameter. Each cell contains a number of starch-grains varying from twelve to twenty. The grains are large, fairly regular in size, nearly circular in outline with an almost central hilum, and measure as much as $45\ \mu$ in diameter. The cells viewed in the fresh tissue are almost transparent, except for the starch grains and a faintly visible nucleus.

If, however, the bulb has been preserved in alcohol, the cells present a different appearance. What in the living cell was occupied mainly by liquid, is now solid, making the tissue quite hard to cut and friable to the touch. The cell is almost filled with a nearly hyaline mass, in which the starch grains are embedded. The nucleus of the cell can easily be rendered visible by staining, and is not enclosed in

the mass, but is either clinging to it, or to the cell-wall. This solid precipitate in the cell is, in fact, the inulin, which in the fresh scale remains dissolved in the cell-sap; on the penetration of the alcohol it is precipitated and takes the form of the cell, in which it is held, enclosing the starch-grains. These casts can easily be liberated from the walls surrounding them, and can be seen under the microscope floating about as isolated masses, holding the starch-grains. If a starch-grain be freed from a cast, then a circular depression remains in it, showing where it was retained.

These inulin masses are not confined to any special part, but, like the starch, occur throughout the parenchymatous tissue of the scale. They are insoluble in cold water and glycerine, so sections exhibiting them can be mounted permanently in dilute glycerine (see Plate 3 for figures).

Successive Microscopic Examinations of Bulbs taken up during an entire year.

These were begun in September, 1896, and completed in August, 1897. The descriptions to be given, however, commence from the time when the foliage leaves had just died down, so as to enter upon the plant at its resting period, if it has one. The dates when the bulbs were taken up for investigation are given, but it does not necessarily follow that the majority of snowdrops were in exactly the same conditions of growth at each of these times, my endeavour being rather to obtain plants at intervals of sufficient frequency, so as to thoroughly work out the changes in the foliar sheaths (scales). The plants, as a rule, were placed in methylated spirit as soon as taken up, and then examined at leisure, thus giving time for the complete precipitation of the inulin. Several bulbs were frequently dealt with at one period. The plants were obtained both from Cambridge and from the north of England, near Carlisle. The measurements given were made of the widest parts of the scales.

(1.) *June 3, 1897.* The leaves about withered (see figs. 1, 2, 4). The old scales (the bases of the foliar organs and scape of the Spring of 1896) are entirely exhausted of starch and inulin, brown in colour, and very thin. Sections of them under the microscope show the two epidermal layers, enclosing the much compressed cell walls of the parenchymatous tissue, which assumes the form of sinuous strands, and amongst them are visible xylem vessels and raphides. The strands take on a blue colour with SCHULTZE's solution, and so are unaltered cellulose.

Outer scale (base of the tunic), 2 millims. thick; full of starch and inulin. Starch, however, is absent for a few millimetres from the top, inulin alone being present here.

Scale 2 (tunicate base of the outer foliage leaf), 3·4 millims. thick; inulin and starch throughout.

Scale 3 (partial base of the inner foliage leaf), 3 millims. thick; similar to Scale 2; encloses the scape base, also with inulin and starch.

In the centre is the bud, which will produce the leaves and flower for the Spring of 1898; it is now very small, not more than 2 millims. high. At this period, when the foliage has lost its green colour and is withering, the bulb-scales have reached their maximum size and gained their maximum contents, the plant may be said to be in its dormant condition, meaning by this the absence of any visible alteration going on in its tissues.

(2.) *July 2.* Foliage died down, but the old roots still fairly fresh looking.

Outer scale. About 2·5 to 6 millims. below the circular top of the scale occurs a meristematic ring of cells;* longitudinal sections through this region reveal only inulin above the active cells, the starch stopping here. Starch was similarly absent from this part in the bulbs of June 3, although no line of demarcation was then visible. In one bulb the part above the zone was depleted of inulin, and inclined to separate off in the middle of the cell-divisions. Sections lower down in the thick part of the scale show starch and inulin throughout, but the starch-grains just within the inner epidermis are smaller than elsewhere, and are probably undergoing dissolution. The inner epidermal cells themselves now possess small starch granules, as shown up by iodine, but the outer ones contain none.

Scale 2. A meristematic zone occurs here likewise 2-3 millims. from the top, no starch being above it (fig. 3). Sections of the thicker part of the scale show some depletion of starch in 3-5 rows of cells below the inner epidermis, least evident in the layer just below (the hypodermal layer as it will be termed). The inner epidermal cells have small starch granules, but not the outer.

Scale 3 and scape base. Similar to Scale 2.

Central bud, about 2·5 millims. high, showing now the rudiments of the foliar organs; the commencement of the development of the flower is just apparent.

(3.) *July 15.* The old roots still fairly fresh looking.

Scale 1. Part above the meristematic zone depleted of inulin and turning brown, the starch coming almost up to it. In the thick part of the scale 2-4 layers of cells below the inner epidermis are devoid of or have very little starch. The inner epidermal cells have larger starch granules than on July 2, but the outer are still without them.

Scale 2. Parts above the zone are turning brown and depleted; 4-5 layers of cells below the inner epidermis show depletion of starch, but the inner epidermal cells all possess starch.

Scale 3. Similar.

Scape base. A little starch in all the epidermal cells; some depletion on the side next the central bud.

Central bud, nearly 5 millims. high. The several whorls in the flower can be made out by means of sections as small protuberances; most starch occurs in the young spathe of the bud, as very small granules.

(4.) *July 27.* Some of the bulbs have their roots still fresh looking, others have them wholly shrivelled up, the old root-plate bearing them being easily rubbed off from the base of the bulb. The parts of the scales above the zones are brown, and easily detached. The features of the scales are similar to those of July 15, with perhaps a little more depletion.

The chief points to notice so far are :—

(a.) The flower for the next year is not visible at the time the leaves die down, as might have been expected, but gradually becomes differentiated from this period.

(b.) The resting period of the bulb must be extremely short or absent, seeing that conspicuous microscopic changes occur between June 3 and July 2.

(c.) The formation of the new meristematic zones below the place whence the foliage part of the leaves have been detached and the depletion of the areas thus cut off, which previously contained only inulin.

(d.) The depletion always begins below the inner epidermis and extends thence.

(e.) The inner epidermal cells contain starch, while the outer do not.

(5.) *August 5.* Depletion somewhat similar to or rather more advanced than on July 27. The

* See PARKIN, 'Annals of Botany,' June, 1898, p. 151, for details concerning this absciss-layer.

central bud was about 6 millims. in height, and in it the anthers could be recognised as colourless pointed structures.

(6.) *August 13.* The new roots just visible after the removal of the outer scale, as small papillæ not more than 2 millims. long; depletion advanced somewhat; the inner epidermal cells of the scales have conspicuous starch granules, which are wholly wanting in the outer central bud, about 6 millims. high, showing now a difference between the outer (sepals) and inner (petals) perianth segments; also the pointed anthers and the style as a very short papilla are visible.

(7.) *September 7.* The new roots are well protruded and depletion has advanced considerably.

Scale 2. The depleted area is occupied by cellulose strands due to the compression of the empty cells; inner epidermal cells with starch, outer ones devoid of it.

Scale 3. Similar.

Scape base has strands of cellulose and depleted spaces in the central part.

Central bud fairly large. Its tunic has plenty of starch throughout as small granules, not more than $10\ \mu$ in diameter, but no inulin. The young foliage leaves have starch only in the lower part; plenty of starch in the bract, perianth, and anther-connectives of the flower.

(8.) *January 8.* The plants taken up had their flower buds well protruded and level with the tops of the foliage leaves, the latter being about 35 millims. above the apex of the tunic, which at first encloses the foliage leaves and scape, increasing in length with them, but soon its growth ends and then it is pierced by the tips of the leaves. The upper part of the tunic which lies above the bulb is membranous, forming a sort of protective sheath to the lower parts of the leaves and scape, where active cell-division and growth are taking place. Its base, of course, swells and becomes the outer scale of the bulb.

Scale 1. The depletion has advanced considerably since September 19, and has kept steadily progressing from the inner epidermis towards the outer. Below the inner epidermis is a band of cellulose strands, due to the internal pressure exerted on the empty cells by the expansion of the new bulb-scales. There are only about six layers of cells below the outer epidermis with starch, the rest are empty. The starch-grains now show much internal fissuring. The vascular bundles are still surrounded by starch-holding cells, and inulin is still present in the cells containing starch.

Scale 2. Starch distributed as in Scale 1; there are comparatively large depleted spaces between the bundles. Inulin not abundant, and the masses precipitated by alcohol do not entirely fill the cells (fig. 5).

Scale 3. Similar to 2.

Scape base contains very little inulin, starch is confined to the cells around the bundles and beneath the epidermis; the rest of the area is depleted.

No starch could be found in the epidermal cells of any of the scales.

New tunic base is much swollen (about .75 millim. thick), and full of starch except in two or three layers of cells below the inner epidermis, the grains as large as $20\ \mu$. Inulin also occurs in it, not throughout, but only in two or three rows of cells round the bundles, so that in transverse sections its distribution is in circular patches. Above the swollen part no starch or inulin seen.

Outer foliage leaf base, 1.12 millims. thick; contains plenty of starch in very small grains; several layers of cells below the inner epidermis without starch.

Inner foliage leaf base, .75 millim. thick; starch less in amount, and even in smaller granules, than in the outer leaf base, and none in some layers of cells below the inner epidermis. No inulin in either base.

New scape base. Starch throughout and fairly evenly distributed.

The further points to notice now are that:—

(f.) New roots begin to be protruded some time after the bulb recommences its

growth, and are probably induced by the greater amount of moisture in the soil about the middle and end of August than in July.

(g.) The starch and inulin are used up together, and not one before the other as might have been expected.

(h.) The tunic base becomes, at an early age, filled with starch in fair-sized granules, and partially with inulin, when the leaf bases are in a much less advanced stage. As the leaves have only been green for a brief time and are still short, one must assume that much the greater part of the carbohydrate now stored in the new tunic base, has come from the depleted areas in the old scales and not from new assimilated material.

(i.) The deposition of starch occurs last in the layers of cells below the inner epidermis of the new scale, this being the region where it first disappears in the old scale.

(9.) *March 6.* Just at the end of the flowering period; the depletion in the old scales somewhat similar to that of January 8.

New tunic base. .9 millim. thick. Starch throughout; least below the inner epidermis. Two to three layers of cells around the bundles contain inulin casts; none elsewhere. Parenchymatous cells about 38μ wide.

Outer foliage leaf base. 1.5 millims. broad. Starch throughout; grains 10μ in diameter. Inulin casts occur in circular areas of five to six layers thick round the bundles (fig. 6).

Inner foliage leaf base. 1.1 millims. in breadth. Similar to the outer leaf base.

New scape base also with inulin and starch.

The new bud is just visible to the unaided eye in the centre of the bulb.

The additional features brought out here are that :—

(j.) The inulin is in process of being deposited in the new scales, and appears to be stored first in the cells round the vascular bundles and gradually extends outwards, although it is just possible (but hardly likely) that the inulin may be drawn to this region during the penetration of the alcohol.

(k.) The starch is first deposited in the scales in considerable quantity before the inulin makes its appearance.

(10.) *March 30.* The old scales resemble those of January 8, as regards depletion.

New tunic base. 1.2 millims. thick. Starch and inulin throughout. Grains as large as 35μ in diameter. Parenchymatous cells as broad as 70μ . A meristematic band is just visible where the membranous upper part will become detached from the lower reserve part, and no starch or inulin occurs above this absciss-layer. The starch comes nearly, and the inulin quite, up to it. Very minute starch granules now exist in the inner epidermal cells.

Outer foliage leaf base. 2.25 millims. thick. Starch throughout, but seven layers or so below the inner epidermis have smaller grains and no inulin; grains 30μ and cells 60μ in diameter. Inulin is not present throughout, but extends some way irregularly around the bundles. There is no starch in the inner epidermal cells.

Inner foliage leaf base. Similar in most respects to the outer.

Scape base. 1.75 millims. thick. Inulin not throughout, but more round the bundles.

(11.) *April 16.* The old scales not wholly depleted, but more so than on March 30.

Scale 1 has three layers of cells below the outer epidermis with starch and inulin.

Scale 2 has one to two rows of cells, below the outer epidermis, with starch; none round the bundles, and a fair amount of inulin partially fills the cells.

Scale 3 similar to Scale 2.

Old scape base. No starch, only inulin.

New tunic base. 2 millims. thick. Full of starch and inulin. Grains full size and cells 90μ wide. Absciss-layer obvious, and for 5 millims. or so below it no starch occurs, only inulin. Starch present in the inner epidermal cells.

Outer leaf base. Starch throughout, as much as 25μ in diameter; rather smaller grains below the inner epidermis, which now has itself minute starch-grains. Inulin almost throughout the scale, but not filling it as it does the tunic base.

Inner leaf base and scape base. Similar.

Central bud visible, but very small.

(12.) *May 1.* Leaves quite fresh and green. Old scales wholly depleted. The new scales somewhat resemble those of April 16, except that the upper part of the tunic is ready to split off, and a layer of cells for the separation of the upper part of the scape from its base is visible; the bulbs on this occasion were not examined microscopically.

(13.) *May 5.* Leaves turning yellow at the tips, but still fairly green. The old scales were now much compressed, but not wholly depleted, and the absciss-layers for the separation of the upper parts of the leaves from their bases (scales) were visible. Central bud still very small.

(14.) *May 10.* Leaves still fairly green. The old scales contain starch and inulin in almost the same quantities as those of April 16.

Tunic base, 2 millims. thick. Starch and inulin throughout, the grains being as much as 38μ , and cells 98μ wide. No starch for 2 millims. or so below the top.

Outer leaf base, 4.5 millims. thick. Starch and inulin throughout, but scarcer below the inner epidermis; a well-formed absciss-layer for the severance of the foliage part has arisen, starch and inulin coming nearly up to it, but not beyond it.

Inner leaf base. Similar.

Central bud not more than 1.2 millims. high.

(15.) *May 25.* Leaves almost completely yellow. Old scales about exhausted and turning brown.

Tunic base (now the outer scale of the bulb). Inulin and starch throughout. Starch is absent for a few millimetres from the top. The scale measures 2 millims. in width.

Outer leaf base (now the 2nd scale) is 3.4 millims. wide, and has starch and inulin throughout.

Inner leaf base (now 3rd scale) and scape base. Similar. The central bud is very small, only 2 millims. in height.

Thus the cycle of growth commenced on June 2 has been completed, and the remaining features to notice are:—

(l.) The inner epidermal cells appear to be the last to deposit starch; the outer ones seem never to possess any.

(m.) The old scales are not completely exhausted till the leaves die down. There is a fairly rapid depletion of them until the leaves are well above the ground, and their condition remains somewhat similar till towards the end of the assimilating period, when they are wholly deprived of their carbohydrates.

(n.) The formation of the absciss-layers.

Bulbs grown in the dark.—It is interesting to compare the changes in the bulbs of

normally grown plants with those grown in the dark. Several bulbs were taken out of the ground in September, and planted in soil in total darkness. They sent up their leaves, which were, however, yellow, narrow, and much elongated, as etiolated leaves usually are; their scapes, also, came up, bearing swollen flower buds, which, however, in no case expanded, or assumed a horizontal or pendent position, but remained erect, and withered away.

(1.) *January 22.* The scales presented much the same appearance as in the case of normal plants of January 8 as regards depletion.

New tunic base, .75 millim. in thickness. Starch throughout, except in three or four layers of cells below the inner epidermis; the grains small, not more than 8μ in diameter, and the parenchymatous cells about 40μ wide. No inulin visible.

Outer leaf base, 1.12 millims. thick. Starch throughout, except in seven or eight layers of cells beneath the inner epidermis, in very small grains, and the cells 33μ wide. No inulin.

Inner leaf base, 1 millim. thick. Similar to the outer scape; less starch, chiefly in the peripheral parenchyma. Thus the foliage leaf bases are in about the same condition as those of normal plants of January 8, but the tunic base has not so much reserve material. In this case all the starch must have come from the scales.

(2.) *April 1.* The three old scales present an almost identical appearance, as regards depletion, as those of normal plants examined on April 16.

Tunic base, .98 millim. thick. Starch uniformly throughout, grains as large as 33μ , and parenchymatous cells 70μ wide. Inulin round the bundles.

Absciss-layer present.

Outer leaf base, 1.8 millim. thick. Starch throughout, but less for a few layers below the inner epidermis; grains as large as 15μ . Inulin in one or two layers of cells outside the bundles, and not as much as in the spathe; cells 44μ wide.

Inner leaf base and scape base. Similar.

Central bud. Very small.

On comparing the above with the darkened bulbs of January 22, it is seen that the reserve material in the new base has greatly increased, inulin being now present; this is accounted for by the greater depletion of the old scales.

Comparing them with normal bulbs of April 16, the latter have the tunic bases twice as thick, their starch grains of maximum size, and inulin in the cells (which are double the size of the former) throughout; all this increase in bulk over the darkened bulbs must be due to assimilation. The leaf bases likewise show a corresponding increase in thickness, and in their contents of starch and inulin. The two lots resemble each other in the small size of the central bud.

Some of the plants growing in the dark were again examined on April 30, but only macroscopically. The leaves were beginning to wither at their tips, and the old scales still contained starch. Unfortunately I was unable to obtain the bulbs growing in the dark after the foliage had completely died down. However, I had one growing in distilled water in the dark, the leaves and scape of which grew about as well as those in the soil, the roots being fewer. The old reserve scales were almost exhausted, and the new scales had much the same appearance as the darkened ones of April 1.

The point which has struck me most forcibly in these observations of the changes in the Snowdrop bulb, is the retarded formation of the flower bud for the ensuing year. One might have thought that the flower bud with its various parts, as well as the leaves, would have been fully differentiated in the central bud by the time the foliage had died down. However, almost all the carbohydrate arising from assimilation, except what is required for seed production and respiratory and metabolic processes, appears to be stored at first in the scales, and then to be withdrawn gradually from these when the plant has died down for the production of new leaves and flowers. Thus it is not evident what causes the formation of a flower in one bulb and not in another, or what calls forth the production of axillary bulbs. It hardly seems to depend on the amount of carbohydrate accumulated; probably it is connected with the quantity of nitrogenous material available in the scales. The crowding together of bulbs is generally looked upon as detrimental to their flowering; this may be due not so much to the shading of each other by the foliage, as to the exhaustion of the soil about them of its nitrogenous and other mineral constituents.

B. *Narcissus Pseudo-Narcissus*, var. *plenus*.

As in the case of the Snowdrop I give short accounts of the morphology and histology of the bulb, gathered from my own observations :—

Morphology of the Bulb.—The axis of the bulb is said to be unlimited in its growth, the floral scapes being borne laterally in the axil of the innermost leaf, as in *Galanthus*. A bulb may consist of as many as fourteen scales holding reserve material, covered by the brown papyraceous exhausted ones. The scales are tunicate and consist of the swollen bases of the foliage leaves, two to four in number, and of the lower parts of the sheathing foliar structures (tunics), usually two, sometimes only one. When a flower is again produced, the innermost leaf has only a partial base, otherwise it is tunicate. New bulbs are borne in the axils of the scales, and are two or even three years old when released from the mother-bulb.

The scales persist from two to three years as reserve organs; the outer ones are very thin with only two to three undepleted layers of cells. In the centre of the bulb is the bud, which contains the foliage leaves and flower for the succeeding year.

From observation it appears that when a bulb sends up a flower it has usually only two leaves, and when without a flower three leaves, rarely four, as may be concluded from the following figures :—

Out of 457 plants examined with flowers, 357 had 2, and 100 had 3 leaves; while of 483 plants examined without flowers, 430 had 3, and 53 had 4 leaves.

The fundamental difference between the morphology of the Daffodil and Snowdrop bulb is that in the former its scales possess food material for two or three years before they are exhausted, while in the case of the latter they are wholly depleted in one year,

Histology of the Bulb.—The structure of the bulb-scale is very similar to that of *Galanthus*. Two epidermal layers enclose parenchymatous tissue holding starch, through which run the vascular bundles and the vertical rows of raphide-cells.

The starch-grains are more numerous, not so uniform in size, less regular in shape, and not quite as large ($33\ \mu$ in diameter) as those of *Galanthus*.

The raphide-cells are larger and more numerous, and the extra quantity of mucilage arising from them makes the bulbs, when cut, very viscid.

Bulbs preserved in alcohol show no inulin casts or granules in the parenchymatous cells, only the hardened mucilage in the raphide-cells.

No inulin could be obtained from the powdered dried bulb-scales by following out the process employed for the isolation of the inulin of *Galanthus nivalis*. Its absence is what might be expected, since sections of the spirit-material do not give the characteristic colorations with the α -naphthol and orcin tests.

The Changes in the Bulb-scales.—Similar investigations have been made on the depletion of the scales and on the storing of the foliar bases at various stages of the plant's growth, as in the case of *Galanthus nivalis*; it will suffice to point out the chief features of resemblance and difference.

The depletion commences similarly to that of *Galanthus* below the inner epidermis, and seems to go on simultaneously throughout the scales; there is, however, a more marked hypodermal layer of cells full of starch, which is rendered conspicuous as the exhaustion of the other cells advances towards the outer epidermis, and is well seen in the half-depleted scales of a year old (fig. 7). The inner epidermal cells may contain starch.

The formation of absciss-layers for the severance of the upper parts of the tunics and leaves occurs as in *Galanthus*, and others are produced a short time after the plant has completely died down, which happens about the end of June. Extra ones are also formed as the scale advances in age and depletion, marking off the upper exhausted part from the lower part still containing starch. Since the upper portion of the scale is narrower than the lower, the depletion proceeds from the top downwards as well as from within outwards.

The resting period of the bulb must be very short or absent, for on July 2 depletion had commenced in the spathe and foliar bases (the new scales of the bulb), the starch grains in the cells below the hypodermal layer being in process of dissolution.

The flower-bud, like that of *Galanthus*, only begins to be differentiated after the plant has died down, and is well formed by the middle of August, when the new roots commence to appear.

A number of bulbs were put in the dark in the same situation and soil as those of *Galanthus*, in the autumn of 1896. They grew well and flowered, producing full-sized blooms, like those of plants grown normally, except that they had of course no tinge of green in them. I allowed most of them to die down, then dug them up in

July, 1897, and replanted many of them. When last observed in February of this year (1898) they had all sent up new foliage leaves, but none of them showed any signs of possessing flower-buds. Some of these bulbs were microscopically examined when taken up in July and showed plenty of starch in their scales. One bulb had eleven scales with starch, including the tunic and foliar bases of the current year. The foliar bases had stored in them a fair proportion of starch, but they were thinner than the normal ones, and the starch-grains smaller. The starch they possessed must have come wholly from the reserve-scales, since the leaves were incapable of manufacturing carbohydrate, being in the dark. Thus it looks as if in the normally-growing plant, part of the starch deposited in the bases of the foliage leaves is drawn from the older scales, and part is derived directly from carbohydrates formed in the mesophyll.

The following question naturally suggests itself from the results of the investigations on these two bulbous plants. Why does the depletion of the bulb-scales always commence below the inner epidermis and continue steadily towards the outer epidermis? If it took place the reverse way, then the cell-walls left might be looked upon as affording a protective cushion to the unexhausted parenchyma within. The course it pursues, however, seems best fitted for allowing the expansion of the foliar bases; the empty cells, being situated below the inner epidermis, will readily yield to the pressure exerted by the increase in thickness of the newly-forming reserve scales. In a dorsiventral leaf, as a rule, the starch during assimilation appears last in the palisade cells—the layers of mesophyll below the upper epidermis—and it is the starch in this region which is the first to disappear during depletion. In the scale it is likewise the layers of cells below the morphological upper surface which are the last to receive and the first to give up their reserve carbohydrate. The correspondence, nevertheless, is not close, for in the leaf-lamina the starch on the whole is deposited relative to the vascular bundles centrifugally, and disappears centripetally, thus remaining longest in the cells around the bundles; while in the scale, on the contrary, the cells which retain the starch the longest are those below the outer (lower) epidermis. It is possible that the enzyme, which presumably is secreted for the hydrolysis of the starch, originates in the inner epidermal cells; if so this would account for the mode of depletion. The presence of starch-granules in the inner epidermal cells and not in the outer may have some significance here. On the other hand it seems far more likely that the enzyme is produced, when required, in the cell containing the starch; for one reason the inner epidermal cells show no distinctive secretory character, and for another reason the starch-grains, when in process of dissolution, show none of the pitting so characteristic of the endosperm-starch of the gramineous seed, which is hydrolysed by diastase secreted by a special epithelial layer of the embryo.

Although no extensive series of analyses of the carbohydrates in these bulbs have been made, yet it is of some interest to point out one marked feature in the character

of the sugars obtained from the bulb-scales of *Galanthus nivalis*, viz., the preponderance of saccharose. The scales were removed from bulbs taken up at the end of September, dried and powdered, then extracted with 90 per cent. alcohol. This extract, containing the sugars, was evaporated down and taken up with water. The aqueous solution gave a slight reduction with FEHLING'S cupric solution, which was increased about eight times after heating with 1 per cent. hydrochloric acid; during the hydrolysis the opticity changed from being dextrorotary to levorotary. Yeast, with a few drops of chloroform added to prevent its fermentative action, increased the cupric reduction and gave a left-handed reading in the polarimeter to about the same extent as after-treatment with acid, thus indicating saccharose as the chief sugar present. Analyses conducted quantitatively gave an average of 11 grams of soluble carbohydrate (calculated as glucose) in 100 grams of the dried bulb-scales. Considering the nature of the reserve carbohydrates, saccharose is hardly the sugar one would expect to find so largely present, but rather maltose and fructose derived from the starch and inulin respectively. This is an interesting point, requiring further investigation. Saccharose seems evidently to be the best nutritive sugar for plants. BROWN and MORRIS* found that barley embryos grew most vigorously when the carbohydrate was supplied to them in the form of saccharose. When maltose was substituted, then, not it, but saccharose accumulated in the embryo, thus signifying a transformation of the former into the latter. Probably some such change takes place in the Snowdrop bulb. Saccharose easily hydrolyses into glucose and fructose, whereas maltose only gives rise to glucose. It is quite possible that growth proceeds best when both sugars (glucose and fructose) are available, each playing distinct parts in the constructive metabolism; the one, glucose, may be more readily used in the building of the cell-wall and in respiratory processes, and the other, fructose, in the synthesis of proteids. BROWN and MORRIS† ascribe the lack of glucose among the sugars of the *Tropæolum* leaf to its being used up quickly in respiration, the fructose meanwhile accumulating.

In conclusion, I desire to express my thanks to Mr. HORACE T. BROWN, F.R.S., for much assistance rendered to me in the way of suggestions during the progress of this research, and for kindly advice as to the arrangement of the results, and especially for supplying me with his methods for the estimation of carbohydrates. I am also much indebted to Professor MARSHALL WARD for advice and assistance in connection with this paper.

* BROWN and MORRIS, "Germination of the Gramineæ," 'Journ. Chem. Soc. Trans.,' 1890.

† BROWN and MORRIS, "Chem. and Phys. of Foliage Leaves," 'Journ. Chem. Soc. Trans.,' 1893.

EXPLANATION OF FIGURES.

PLATE 3.

The drawings are enlargements from sections viewed under Zeiss D. objective.

- Fig. 1. Parenchymatous cell in transverse section of bulb-scale of *Scilla nutans*, preserved in alcohol. *i.*, inulin deposit lining cell-wall. *c.*, empty space in the centre.
- Fig. 2. Parenchymatous cell in longitudinal section of bulb-scale of *Galanthus nivalis*, preserved in alcohol. *n.*, nucleus. *i.*, inulin cast in which are embedded the starch-grains, S.
- Fig. 3. Fresh parenchymatous cell of bulb-scale of *Galanthus nivalis*. *n.*, nucleus. S., starch-grains.
- Fig. 4. Isolated inulin cast of *Galanthus nivalis*. S., starch-grains. *c.*, cavity left by a starch-grain which has fallen out.
- Fig. 5. View of a parenchymatous cell from longitudinal section of the fresh bulb-scale of *Galanthus nivalis* after being placed in alcohol. S., starch-grains. *i.*, inulin precipitated in spherules.
- Fig. 6. Cell similar to that of fig. 2, but after treatment with saliva. *i.*, inulin cast with cavities, *c.*, in it left by the starch-grains, which have been dissolved out.
- Fig. 7. Parenchymatous cells from a transverse section of bulb-scale of *Triteleia uniflora*, preserved in alcohol. S., starch-grains. *i.*, inulin deposit lining the cell-wall. *c.*, empty space.
- Fig. 8. Parenchymatous cells from a transverse section of the rhizome of *Yucca filamentosa*.
 (a.) Cell from material preserved in alcohol. *i.*, inulin deposit. *c.*, central empty space.
 (b.) Cell from spirit material, after placing in cold water, showing nucleus, *n.*, inulin deposit has disappeared.
 (c.) Cell from fresh section after placing in alcohol. *i.*, inulin precipitated in spherules.
- Fig. 9. Parenchymatous cells from the rhizome of *Polygonatum multiflorum*, preserved in alcohol. *i.*, thick inulin lining to the cell-walls. *c.*, cavities in the centre free of inulin.
- Fig. 10. Parenchymatous cell from the tuber of *Phleum pratense*, preserved in alcohol. *i.*, inulin deposit completely filling the cell.

PLATE 4.

Figs. 1-3 are diagrammatic and about natural size.

Figs. 4-7 are semi-diagrammatic, under Zeiss B. objective.

Figs. 1-6 belong to the bulb of *Galanthus nivalis*.

Fig. 7 belongs to the bulb of *Narcissus Pseudo-Narcissus*, var. *plenus*.

Figs. 1 and 2 represent respectively transverse and longitudinal sections through the bulb, about July. *a.*, outer scale (base of the tunic). *b.*, second scale (base of the outer foliage leaf). *c.*, third scale (base of the inner foliage leaf). *d.*, lower reserve part of the scape. *e.*, new central bud, containing the rudiments of flower and leaves for next spring. *r.*, new roots. (The old exhausted scales are not represented.)

Fig. 3. Reserve scale formed from the basal part of the outer foliage leaf, at the end of May. *a.*, represents the region where the upper foliar part, *f.*, has become detached by the formation of an absciss-layer. *b.*, represents the second absciss-layer, which is formed a little later.

Figs. 4, 5, and 6 represent parts of transverse sections of the reserve base of the outer foliage leaf at different periods of the year. In each case *eo.* signifies outer epidermis; *ei.* inner epidermis; *p.* phloem, and *x.* xylem of the vascular bundles; *r.* raphide-cells, and *c.* the parenchymatous tissue which stores the carbohydrates.

Fig. 4. From a bulb taken up on June 3. The parenchyma is full of starch and inulin. The starch is represented as small round bodies in the drawing. The inulin is not depicted.

Fig. 5. From a bulb taken up on January 8. The scale now appears about half-exhausted of its starch and inulin. The depleted area is wholly confined to the inner half. *S.*, cellulose strands due to the compression of the cell-walls. *l.*, empty spaces left between the vascular bundles due to the collapse of the cell-walls to the sides. Starch represented as in fig. 4. Inulin has the same distribution as the starch.

Fig. 6. From a bulb taken up on March 6. Section of new scale cut from spirit material, showing starch deposited in all the parenchymatous cells and inulin (shaded areas) in two or three layers of cells round the vascular bundles.

Fig. 7. Transverse sectional part of a one-year-old scale of *Narcissus Pseudo-Narcissus*, from a bulb taken up in July. *S.*, cellulose strands resulting from the compressed walls of the cells depleted during the last vegetative period. *d.*, cells in which the starch-grains are beginning to dissolve. *h.*, hypodermal layer of cells still with starch. Other letters as in figs. 4-6.

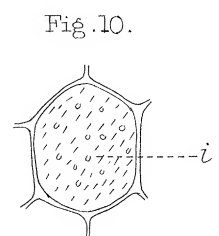
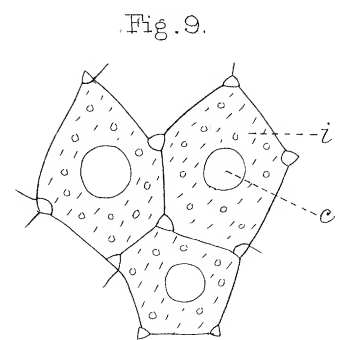
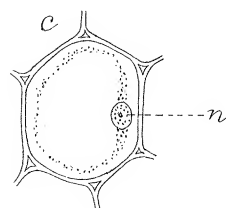
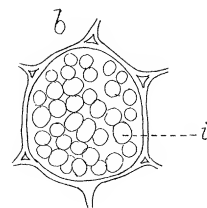
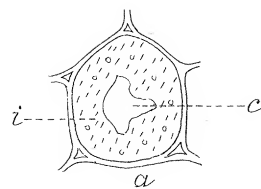
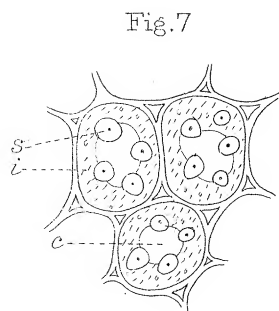
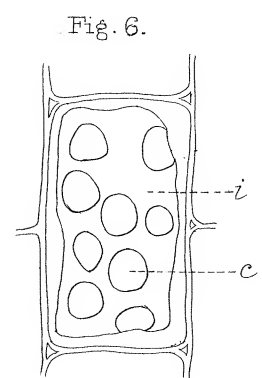
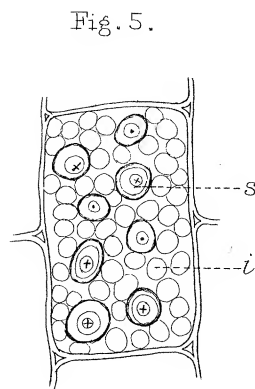
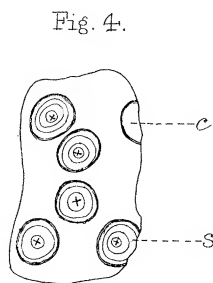
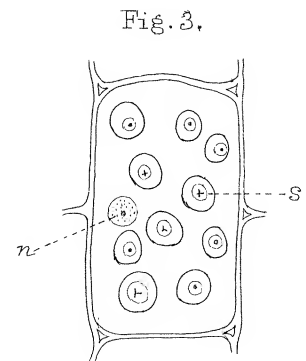
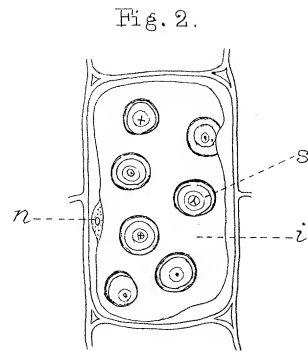
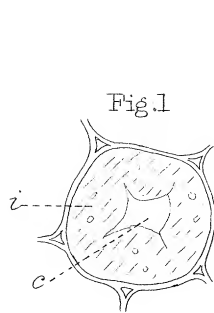


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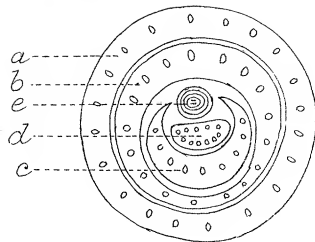


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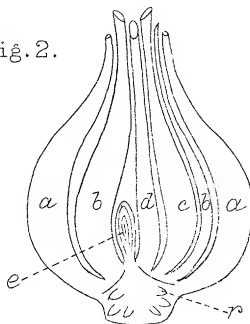


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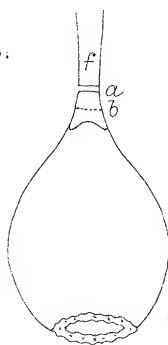


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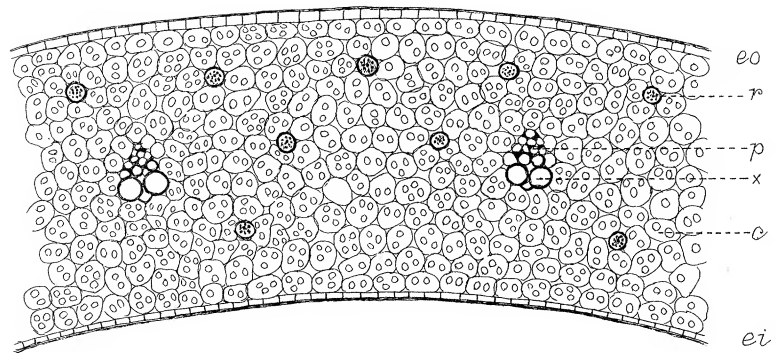


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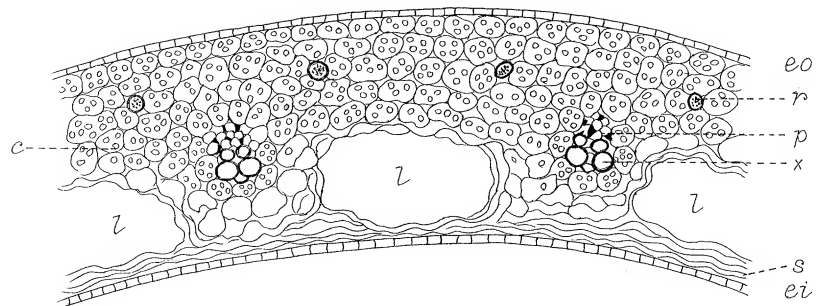


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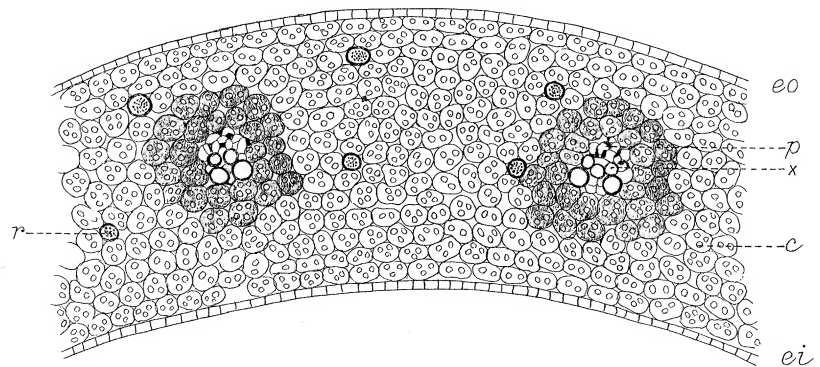
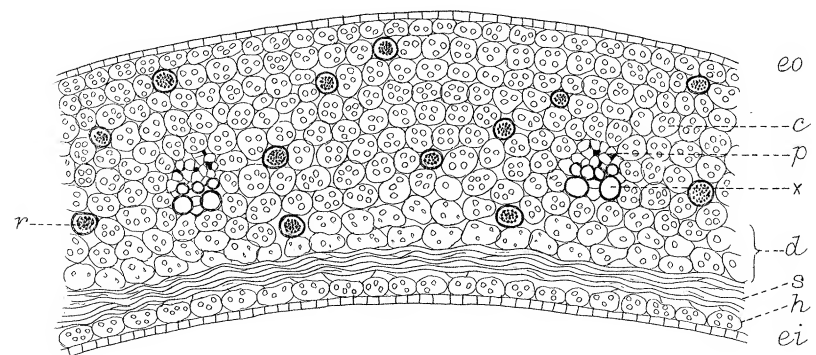


Fig. 7.



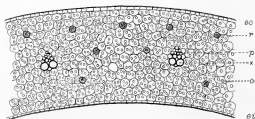


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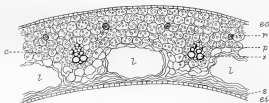


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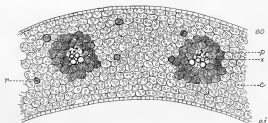


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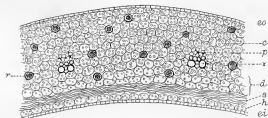


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